

## Ontario Agricultural College and Experimental Farm

## ROUP: AN EXPERIMENTAL STUDY.

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One of the most important poultry diseases in America is fowl diphtheria, more often called "Roup." It is very widespread; but it is more prevalent in some places than in others; thus, for example, it is said to be almost unknown in the Canadian Maritime Provinces and in Eastern Ontario, while in Southern Ontario it is the most prevalent disease of fowls.



Fig. 1.—A section of false membrane of roup fowl (No. 9). *a*, The false membrane partly separated from the middle epithelial layer. *b, c*, Submucosa. *d*, Normal mucous glands.

The views of poultry raisers as to the origin of roup differ very much. A small number trace it to a neglected case of ordinary cold or catarrh, basing their belief on the fact that among fowls living under hygienic conditions,

where even common colds receive prompt attention, roup is quite unknown ; but by far the greater number of breeders consider roup a more or less infectious disease, which is said to be produced by a certain micro-organism. This view is strengthened by accounts of the disease in the literature of poultry breeders, according to which epidemics have been caused by diseased fowls being brought into healthy flocks.

There is also an impression among poultrymen that fowls that have once overcome the disease possess a certain degree of immunity.

In America, V. A. Moore is the only person who has examined roup scientifically. The results of his investigations, however, are insufficient to establish the etiology of roup, and hence the need of further research.

For several years in succession roup has made its appearance in the poultry yards of the Ontario Agricultural College, usually during the cold, damp weather of late autumn and early winter. It usually causes a direct loss of from 10 to 15 per cent., and a much larger indirect one from the disease becoming chronic. It often lasts for months, and makes the fowls attacked absolutely worthless either for table use or for breeding ; and the most valuable fowls of the special breeds are the ones most frequently attacked by the disease. Young birds, six months or a year old, are particularly susceptible.

#### CLINICAL SYMPTOMS.

In the majority of cases, the first symptoms of roup appear in the nostrils. Moore found the disease most frequently localized in the eyes ; while, according to the statements in German, Italian, and French literature, the mouth, pharynx, nostrils, and wind-pipe are the places in which the first symptoms of the disease usually appear.

Over 300 cases of the disease have been studied at this institution. Thirty-six of the most severe were examined daily for several months. Of these, four recovered ; and the others died, or were killed for dissection.

#### Nose.

Nose. Roup usually appears first in weak fowls as catarrh of the nose. At one or both nostrils a serous fluid is observed. Occasionally on a cold night in autumn or early winter a large part of the flock is suddenly attacked by catarrh ; and next morning as many as three-fourths of the fowls show a serous nasal discharge. In from three to eight days many fowls under normal conditions recover from this catarrh, without any further result. In others, however, both the general and local conditions grow worse, and develop through all the different stages of roup.

The serous nasal secretion soon becomes streaked with grey ; a slimy matter forms and dries quickly to dirty crusts, which often completely close the nostrils. The secretion never becomes yellow, as, according to Zuern and Friedberger-Froehner, is said to be the case in European fowl diphtheria.

On the removal of the crusts around the nostrils, a few drops of grey secretion flow out. These can be increased by pressing the nostrils. In later stages, small, solid, yellowish-white particles of matter are often found

in the grey nasal secretion. The secretion decomposes and emits an offensive odor. On the mucous membrane of the nostrils, small cancers form; or the whole nostril is completely filled with a firm, whitish-yellow cheese-like mass, which frequently grows very rapidly and separates the nostrils and forces the dorsal wall of the nostrils upward. This mass soon becomes dry and brown on the outside; and it adheres quite firmly to the mucous membrane. If it is removed, it quickly forms again.



Fig. 2.—A section through the deeper epithelial layer under a firmly attached false membrane. *a*. Swollen epithelial nuclei. *b*. Epithelial layers dislodged from their normal position. *c*. Submucosa infiltrated by leucocytes. *d*. Homogeneous fibrinous exudate. *e*. Mixed fibrinous and purulent exudate.

As the changes above described progress, it becomes impossible for the bird to breathe through the nostrils, and the beak is kept open for breathing. In the early stages of the catarrh, the irritation causes sneezing; but later this symptom disappears.

#### EYES.

From the diseased mucous membrane of the nostrils, the inflammation easily spreads to the mucous membrane of the mouth, pharynx, and larynx; yet, these parts may be the first seat of the infection. When the disease affects the eyes,

there is often formed in the inner corner of the eye a viscous, lacrymal secretion, which contains air-bubbles. These bubbles come from the nose, and are forced into the eye through the lacrymal duct, because the natural air-passageway through the nostrils is half stopped up and the air finds this outlet.

Soon a serous Conjunctivitis forms. The Conjunctiva becomes very much swollen and grey. The secretion gradually assumes a slimy, purulent character. The lids swell oedematously, and become perceptibly thicker in a short time. They are hot, sore, very much inflamed, and stick together easily, because the eye at this stage of the disease is, for the most part, shut. Often the lacrymal duct remains open, and the secretion passes into the nasal or pharyngeal cavity. In all the more severe cases, however, this canal is completely closed. The accumulating secretion frequently overflows into the inner corner of the eye, and defiles the surrounding region with greasy crusts of dried secretion. The small feathers on the side of the lids stick together, and often fall out. When the lids stick together, they are forced outwards by the masses of secretion formed under the eye. The secretion consists of a somewhat thin, clear, or turbid fluid, containing jelly-lumps, which are clear or striped with grey. The gelatinous masses are formed by a homogenous, or lightly striped, unstainable substance, mingled with pus corpuscles, epithelial cells, and bacteria. The grey parts are much richer in leucocytes than the clearer ones. The epithelial cells are ciliated with or without a swollen unstainable nucleus. The free epithelial nuclei are usually very much swollen (as large as 16m.m.), round, homogenous, slightly granular and unstainable. In the centre of the nucleus there may be found one or two small, round bodies which can be stained.

If the secretion is left in the closed eye-lid, it may be completely changed in 24-48 hours to a firm, smooth, yellowish-white, cheese-like body, which fills up the whole eye-lid, and lies like a cap over the bulbus oculi. This cheesy mass may become so large that it forces the lids open, and projects between them. The outside then dries and becomes a brownish crust. Moore explains the formation of these cheesy masses by assuming that the liquid content of the eye-lid undergoes coagulation; but, as stated above, they (the cheesy masses) are formed from jelly-like masses in the eye-lid. In all probability, the secretion is an abnormal product of the lacrymal glands and conjunctiva, and contains epithelial cells, as well as free swollen epithelial nuclei.

The grey spots of these gelatinous masses contain very many round cells. The greyer, firmer, and more turbid this mass becomes, the more numerous are the cells. When the mass finally assumes the cheese-like appearance, it consists of leucocytes, granular detritus, epithelial particles, and bacteria. The yellow cheese-like masses are produced by the pus corpuscles exuding in large numbers on to the surface of the mucous membrane, where they stick together, mix with the pathologic secretions, and probably with coagulable plasma. If the gelatinous masses are removed from the eye-lid and allowed to dry, they become a dirty, grey crust.

It is astonishing with what rapidity cheesy masses that have been removed form again. Thus, from hen 11, cheesy masses, the size of marbles, were

moved from the same eye on three successive days. These solid masses can be easily removed; but they may sometimes adhere to a croupous membrane on the mucosa.

In the further course of the Conjunctivitis, small croupous membranes appear on the Conjunctiva, and on the swollen lids, or the mucous membranes. These usually adhere closely; and, on their removal, the mucous membrane begins to bleed.

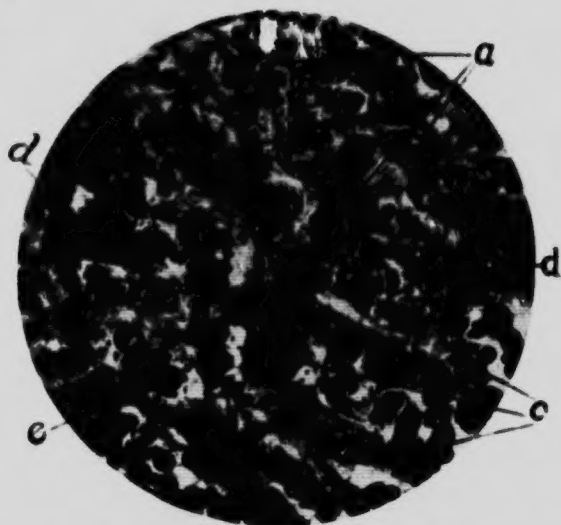


Fig. 3.—Section through the deeper epithelial layer under a firmly attached false membrane from hen No. 9, magnification 700.  
a. Swollen epithelial nuclei with 1 or 2 well-stained nucleoli.  
b. Nucleus of normal size granular staining. c. Leucocytes and fibrinous exudate. d. Granular detritus. e. Two short bacteria.

The Cornea may also become a direct seat of the disease, in which case small croupous membranes form on it. These may remain on the Cornea, or spread over the Conjunctiva of the bulbus oculi. Often the membrane grows through the whole Cornea. If it falls off, the interior eye cavity is either directly opened, or the remaining layer of the diseased Cornea is too thin to resist any following perforation. After the opening of the eye-cavity, a prolapse of the lens may occur; and following this, a purulent ophthalmia develops. After the destruction of one eye, the other usually becomes sympathetically diseased. If both eyes have been originally the seat of the disease, panophthalmia may form on both sides.

The swellings in the eye-lids may remain the same for a long time; but, frequently, the swelling spreads under the inner corner of the eye towards the nostrils. The lids, as well as this tumour, have a high temperature; and are at first soft and oedematous. Later, indurations form in the centre, which become larger and larger, and at last lie directly under the skin. The outside of these indurations is rough or smooth, and the skin on it may be moved. In rare cases, an abscess forms instead of the induration.



Besides the indirect affection of the eyes from the nostrils, there is a more direct way which is also very common. In such a case the cella infra-orbitaria is generally affected from the beginning of the rupy, nasal catarrh, and retained secretions become so large that the bone-walls are pressed out in different directions. Later, these, under the ever-increasing pressure, become absorbed in some places; as, for example, between the inner corner of the eye and the nostril. Here a hot tumor with a hard centre forms. The swelling quickly extends to the eye-lids, after which serous putrid, croupous-diphtheritic conjunctivitis sets in. These tumors are well known among poultrymen, for they frequently recommend the opening of the tumors and "extracting the roots of the disease." The masses taken from the tumors form again, with great rapidity and obstinacy. Often, after there had been for an indefinite period only a slight swelling on the lids, even without conjunctival catarrh, blindness ensued without any preceptible cause. Later, the iris and pupil became grey, and the cornea cloudy. When the disease of the eye appeared in this way, the general condition was always very much disturbed. In these cases, the post-mortem examination showed that the phlegmonous processes had spread from the eye-lids into the loose tissue of the peri-orbital cavity and grey, streaked pus, or thick, well-marked, yellowish-white, cheese-like lumps of matter, like those in the eye-lid, were found.

#### MOUTH AND PHARYNX.

Mouth and Pharynx. Primary or secondary catarrh of the nose, croupous or diphtheritic membranes appear on the mucous membranes of the mouth and pharynx, especially about the fissure of the palate, on the hard palate, on the side of the mouth, near the larynx, and under the tongue. They are usually small, but they often grow to large, extended patches. The thickness varies from  $\frac{1}{2}$ -4 m.m. The outside is uneven or smooth. The color is yellowish-white; in the older membranes, a light brown. They either adhere firmly, or fall off easily. In the former case, they leave a dirty, greyish mucous membrane which bleeds slightly; in the latter case, the underlying mucous membrane appears unchanged. A slight reddening of the region of the mucous membrane affected, may precede the appearance of the pseudo-membranes. Usually, however, this is not the case; but the mucous membranes is pale, with a grey surface, which can be easily removed as a thin membrane. This grey superficial membrane gradually changes to a croupous or diphtheritic membrane.

In fowls 1, 4, 9, and 10, the first stages of a diphtheritic membrane were present, and the ducts of the sub-lingual glands were filled with a clear, tough, gelatinous secretion, which was turbid, and which finally became a solid yellowish-white membrane. At first, these membranes were arranged in lines on both sides near the tongue. Later, they spread farther and became a single large membrane. In addition to this, in fowl 9, there developed a large tumour in the submucous tissues, which contained a firm, cheese-like lump directly adherent to the membrane. Frequently large swellings in the region of the sublingual glands occurred, which then hardened to firm, smooth, or uneven

tumours, while the mucous membrane of the mouth remained apparently normal. These tumours enclosed the above-mentioned cheese-like masses.

The pseudo-membranes in the mouth may appear in a third way, which usually happens in more severe types of the disease. The *Cella infra-orbitalis* becomes distended on all sides by the secretion retained in it. For this reason, it presses the palate towards the mouth-cavity. The palate, therefore, may thus attain twice and three times its regular breadth (as in fowls 5 and 27). Where the pressure is strongest, the bone stratum becomes absorbed, and a typical pseudo-membrane forms. This latter may easily be removed; and if left it communicates directly with the firm cheese mass in the infra-orbital cavity.



Fig. 4.—Section through a firmly attached false membrane from hen No. 17. *a*. An altered epithelial nucleus not quite in focus, and consequently, not sharply outlined. *b*. Nuclei of the leucocytes. *c*. Granular detritus. *d*. A number of bacteria in the middle of an homogeneous fibrinous exudate.

In fowls 14 and 17, in addition to diphtheritic membranes on the mouth near the angle of the beak and at the nostrils, tumors were formed on the skin. These grew to the size of a pin head, or the pit of a cherry. The feathers on the tumours fell out, and the skin beneath was found to be covered with grey scales. The tumours were in direct connection with the pseudo-membranes on the mucous membranes, and formed a grey, dry, crumbly mass.

While in the cases under observation, the region around the larynx was very often the seat of croup-membranes, the disease never passed into the larynx or the trachea. Yet, according to the statements of many others, these organs are favorite places for the development of pseudo-membranes in the course of American Fowl Diphtheria.

Severe pneumonic dyspnoea appeared in fowls 1, 6, 12, 18, and 19. The thoracic walls were used for inspiration and expiration. The beak and larynx were always kept open. The visible mucous membranes, the crest, and the wattles became cyanotic. The dyspnoea grew worse and worse, and led to suffocation in 1 to 4 days.

In fowls that had been sick a long time, catarrh and inflammation of the bowels set in (hens 3, 4, 13, and 18). The fowls then stopped eating altogether. A severe, putrid diarrhoea followed; the feathers around the cloaca became smeared and matted with greyish-yellow, fluid excrement; and the fowls soon became as thin as skeletons, and usually died from loss of strength.

#### GENERAL SYMPTOMS

Corresponding to the polymorphic local appearances of roup, the general symptoms are also very different. As long as the disease exists only in the form of simple catarrh of the nose, slight affections of the mucous membranes of the mouth, and of the lungs, the general condition is quite normal. In all chronic, or more severe cases, especially after the appearance of swellings on the face and eye-lids, the general condition is disturbed. The sick fowls become weak, separate themselves from the other birds, and cower in a corner

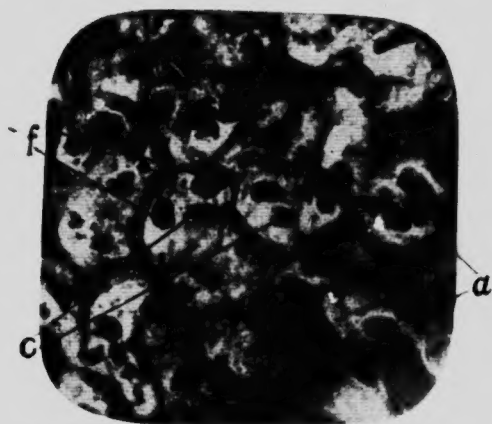


Fig. 5.—Section through the newly formed diphtheric membrane from hen No. 28. *a*. Swollen epithelial nuclei which stained but slightly. *b*. Remains of epithelial nuclei associated with Kitt's "Molluscum bodies" in the epithelial cells.

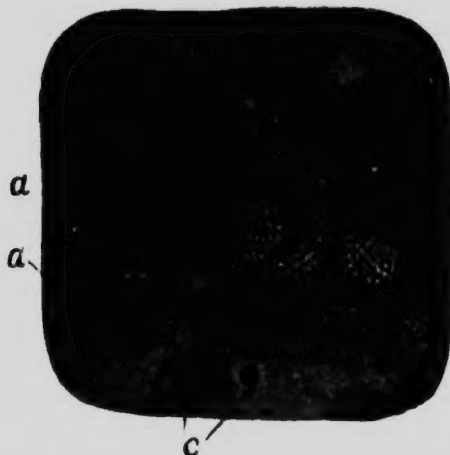


Fig. 6.—Cover-glass preparation from the upper epithelial layer under a false membrane of hen No. 18 stained with methylene blue. *a*. Much swollen epithelial nuclei with or without nucleoli. *c*. Nuclei of leucocytes.

of the yard with head drawn close to the body. The eyes are kept shut, and the head is often drawn under the wings, so that the feathers are smeared with the secretions and fall out. The birds often wake up from this sleeping position, rise, open their eyes, shake themselves, scratch the swollen places with their feet, walk around a few steps, and take some food or water, and sink back again into their apathetic condition. Usually the disturbance of the general condition fluctuates, that is, the birds for a few days often appear in better condition, or normal, and then become worse again. Towards death, they lie down on the floor of the cage and usually do not get up again.

At first, the appetite is not impaired; later, it fails, but often continues for a long time. The taking of food may become quite impossible from purely



mechanical reasons. For example, with fowl 5, the lower mandible was dislocated by the cheesy mass in the nostrils and infra-orbital cavity, and could not be closed. In many cases, there is a pronounced thirst. The fleshy condition of fowls attacked by roup usually becomes worse with the chronic continuance of the disease. The flesh of fowls which die after a short attack, is less affected. As for the body temperature of birds with diphtheria, it is the universal statement that the body temperatures are high in diseased birds. The English and American text-books on poultry all speak of fever as the constant accompaniment of roup. But, according to our observations, their statements on this point are incorrect; and our conclusions are confirmed by the investigations of Friedberger-Froehner. The temperature of the head is very often above normal; but in numerous cases in which we have taken the body temperature of roup birds, there has been no increase; or at least, no considerable increase. The highest temperature that we ever found was 42 degrees C. in a perfectly healthy hen. The minimum temperature of healthy, as well as diseased, fowls, was 40.2-40.8 degrees C., the average being 41.5-41.8 degrees C. In all simple catarrhs of the nose the prognosis is a favorable one. As soon as complications set in it becomes bad.



Fig. 7.—Fowl No. 47 inoculated with *B. pneumatica*.

The course of roup, with few exceptions, is chronic; it lasts weeks, and even for years. Certainly the cause of its becoming chronic lies for the most part in the condition of the exudations or diseased secretions and their localization. Slighter affections of the nose very frequently disappear. If, however, the conchae or the infra-orbital cavities are attacked, or the nose passages become filled with solid masses of exudation, a natural recovery is either impossible or very slow. As has been described, these firm exudation masses become very large in a short time; they act like foreign bodies, setting up irritation, and become larger and larger by the continued transudation; and, by reason of the pressure of these bodies, various kinds of hypertrophies and atrophies ensue. They can be discharged only after having become softened and

liquefied; and such cases appear but seldom, while the cheesy matter in the nose, as well as under the skin, shows no inclination to become so. They, therefore, remain where they were formed, and lead to chronic catarrh. Often there is apparent recovery, so that for a few days or weeks, the clinical symptoms vanish. Then the catarrh again becomes manifest. These cases of apparent recovery are very important, as birds in this condition spread the disease if put among healthy ones. The pseudo-membranes in the mouth and pharynx may be of a very transitory nature. They appear first here, and then there, and, but seldom disappear permanently, so long as any of the other



Fig. 8.—Pigeon (No. 6) 13 days after inoculation with culture of the Roup bacillus, (*B. caecus*) and two days before death.

symptoms of roup are present. In some cases (hen 28), the pseudo-membranes form again with great obstinacy in a certain spot. In these cases, poultrymen describe the trouble as cancer of the mouth. In a few exceptional instances, death occurs after a short sickness. Hen 9 died in this way after symptoms of roup had been recognizable for only two days. This bird showed a strong, hemorrhagic inflammation of the mucous membrane in the olfactory region, as well as swollen vessels in the meninges. Usually, however, death does not come until the disease has run for some time. The direct causes of death are anaemia, suffocation, inability to take nourishment, inflammation of the brain from the nose or eyes, invasion by decaying products of albuminoid substances, and intoxication with bacterial poisons.

#### PATHOLOGICAL ANATOMY.

In addition to the pathologic-anatomical changes already cited in the clinical account, the post-mortem examinations furnish other data. In diseased hen 9, mentioned above, the nasal passages were completely closed by dry, cheesy masses, without any apparent clinical symptom. The bird had been sick two days and showed small bloody extravasations in the upper parts of the nasal mucosa. The mucous membrane itself was very much inflamed.

The eyes were quite normal. The cheesy mass must, in this case, have formed very quickly, and without being preceded by serous-purulent catarrh. This, however, was the only case in which there was hemorrhagic inflammation. The diseased mucous membranes were, in other cases, always swollen; but dirty grey, or brownish in color. They were covered with a grey, putrid mass. Sections through the mucous membrane showed the epithelial cells with cloudy, or finely granular, protoplasm. The nuclei were, in most cases, normal; some were swollen, homogenous, and difficult to stain, excepting one or two extra round central corpuscles, which stained deeply. Many leucocytes were present between the epithelial cells. They were usually rather long in shape,



Fig. 9.—Head of fowl 36, 22 days after inoculation with a culture of the Roup bacillus. *a*. Firmly adherent thick diphtheritic membrane upon the conjunctiva of the upper eyelid.

and were found migrating towards the surface. The sub-mucous tissue showed a numerous increase of leucocytes, and dilated vessels. The leucocytes which migrated to the surface of the mucous membrane became free, and often joined the firm, cheese-like bodies which have been described. As these grew very quickly, they produced various deformities.

The mucous membrane may disappear entirely, or be changed to a brownish tissue of round cells, which are identical with the pyogenous membrane enclosing the cheesy matter in the sub-mucous tissues, etc. In one case (diseased hen 1), part of the mucous membrane of the nose was dry and necrotic. It consisted of a yellowish, dry, crumbly mass, which easily fell away from the cartilage. The latter was itself necrotic. Very often the medial wall of the infra-orbital cavity was absorbed, and then the solid mass of cheesy matter was in direct connection with that in the free nasal cavities. For this reason, the cheesy mass appeared to have grown fast, which, however, was not the case.

In fowl 28, a large part of the solid cheesy matter in the infra-orbital cavity became softened and semi-liquid. The surrounding membrane had changed into a red granulation tumour, which was full of vessels and projected into the cavity. It was lumpy, very soft, and contained in its depths an epithelial layer which grew toward the outside. The conchae of the nose may have been altogether absorbed under pressure of the solid cheesy mass.

The inflamed conjunctiva behaved like the mucous membrane of the nose, except that pseudo-membranes were more frequently formed on them. In the submucous tissue of the eye-lids, which had been seriously infiltrated at



Fig. 10.—Fowl 46; throat and bottom of the mouth with false membranes (*m*), 14 days after inoculation with *B. pyocyaneus*.

the beginning, small, grey bodies of matter were found; these grew large, united and finally became compact, cheese-like yellow-white tumors. The surrounding tissue was sharply separated from these by a greyish-brown, smooth, pyogenous membrane, 1-3 m.m. thick. Usually the pseudo-membranes of the conjunctiva which at first were easily detached, were later firmly fastened to the cheesy masses in the depths of the lids. The firm, cheese-like masses in the lids showed no inclination to become soft, or to perforate the skin, like those formed in other places. On the inner and exterior sides of the lid of infected fowl 35, pseudo-membranes were formed, which had grown fast to one another. Similarly, the whole cornea of fowl 11 and inoculated pigeon 16 were

changed to a diphtheritic membrane. If putrid panophthalmia appeared, the bulbus oculi shrank; the corpus vitreum became grey with pus, and partially softened; and the retina was in shreds.

In birds that were suffering clinically from pneumonic disпноea, and often in those that had not shown any symptoms of disease of the lungs, the post-mortem examination revealed extended pneumonic patches.

In the region of this inflammation, the branches of tracheae were stopped with firm, yellowish-white masses of exudation. Often in the centre of the pneumonic region, a mass of cheesy matter (from 1-2 cubic centimetres in size) was found connected with the masses in the branches of the trachea. These masses of cheesy matter were separated from the lung tissues by a brownish-



Fig. 11.—Head of hen 35, 8 days after inoculation with a culture of the Roup bacillus. *a*. Cheesy matter projecting from between the soiled and featherless eyelids. Nostrils are closed by a crust of dried secretion.

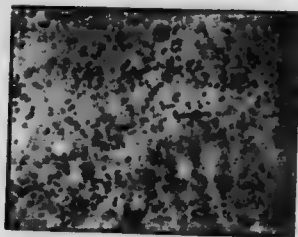
red granular membrane. The more peripheral parts of the diseased lungs were usually normal, or oedematous. The changes in the lungs were often accompanied by cheesy exudations into the pleural folds between heart and lungs. These grew to 3 cm. in length and were often 1 cm. in thickness and breadth. They were always covered by pleura. The latter, however, changed to a brownish, pyogenous membrane, from 1-5 millimetres thick. It was smooth on the outside, but the side, turned towards the exudation, was somewhat uneven, and covered with gray pus. Twice in the pericardium (diseased fowls 6 and 18) a somewhat grey, serous-purulent exudation was present. The pericardium and epicardium were gray. Firm, cheese-like exudation masses were often met with in the folds of the mesentery, between the intestines, gizzard and stomach. In fowls 4 and 18, the mucous membranes of the intestines were haemorrhagically inflamed.



The heart blood was always well coagulated, the spleen small and normal, the liver normal or enlarged, with fatty degeneration.

#### CROUPOUS AND DIPHThERIC MEMBRANES

Croupous and Diphtheritic Membranes. The pathologic anatomical conditions of the pseudo-membranes in mouth, eyes, etc., varied considerably. The small, grey, thin pellicles, which are often the first stage of the membranes, were found to be the outer epithelial-layers mingled with leucocytes. Sections of the epithelial-layers showed these to be more or less invaded by leucocytes, while the submucous tissue usually seemed intact. In all more severe cases, however, the loose, submucous tissue was completely filled up with leucocytes. The epithelial layer underwent changes, especially in the middle layer. The epithelial cells were dislodged from their normal position by fibrinous putrid exudation (Fig. 2). They lost their contour, and the protoplasm became



Figs. 12 and 13.—The Roup bacillus (*B. croupalis*) magnified about 850. The bacillus is stained with gentian violet, the flagella by Van Ermergen's method.

cloudy or decayed to a granular detritus. The cell-nuclei swelled till they became two to six times their normal size, and the contents of the nuclei became homogenous and were difficult to stain, except one or two round, central or excentric nucleoli.

Often, too, the plasm of the nucleus became a fine granular detritus. These granules could then be deeply stained. The abnormal nuclei remained round and sharply outlined by thin nuclear membranes. They appeared like foreign bodies, and have usually been pronounced to be protozoa. Fig 3 shows these transformed nuclei, in which the edges of the separate cells have completely vanished and changed to a partly granular, partly turbid, decayed mass. In these cells there were a few nuclei of normal size; (a) the greater number, however, were swollen in varying degrees, with one or two well stained nucleoli; (b) the contents of the swollen nuclei, when freshly examined, were found to be homogeneous (see Fig. 6); but in Fig. 3, they appear granular, which is due to the treatment of the section while hardening was going on. In (c) we see leucocytes; at (d), two bacteria. The swollen epithelial nuclei are also distinctly visible in the markedly changed middle epithelial layers of Fig. 2 (c).

The swelling might be caused by the simple coagulation of the protoplasm, following death; or there was a mucous or colloidal degeneration. The latter was so much the more probable, as changed nuclei were found in the gelatinous

mucous masses in the eye and in the glanular canals. A certain interchange, therefore, seemed to exist between the two. The transformed nuclei were fairly resistant, which might be due to the condition of the nuclear membranes, for, while the cells themselves decayed, the nuclei lasted for a long time. They were constantly found in all more severe cases of pseudo-membranes. Transformed nuclei might be seen in the slimy-putrid secretions of mucous membranes, in the cheese-like exudations in the nostrils, eyes, bronchial tubes, lungs, pleural and peritoneal folds; or, in other words, wherever there were epithelial or endothelial cells. They were not found in the solid masses of matter in the lids and in other submucous or subcutaneous tissue.

In the above-mentioned jelly-like exudations, the products in the eyes of fowls 35, 36, etc., the epithelial nuclei were greatly swollen. They were homogeneous, clear, with or without nucleoli.

While the changes described in the middle epithelial layer were taking place, compact masses of pus were deposited over this layer and the pseudo-membranes were thus formed. All membranes which could be easily removed became separated from the region of the middle epithelial layer (Fig. 1). We then had typical croup membranes and a regeneration of the epithelium took place from the lower layers. In those cases in which the membranes were firmly attached, even the lowest epithelial layers were very much altered. They were swollen and loosened by fibrous purulent exudations. Under the lasting invasions of the leucocytes, the constituents of the epithelium were lost. The compact mass of epithelial pseudo-membrane advanced towards the lower layer of the epithelium, and finally reached the submucous tissue and thus became a typical diphtheritic membrane.

Submucous glands were often partly, or altogether filled with leucocytes. In typical diphtheritic membranes small quantities of fibrous exudation were found especially in the region of groups of bacteria. The croup-membranes, the cheese-like bodies in nostrils and eye-lids, in bronchii, pleura and peritoneum, as well as the firm masses in the different tumors of the head, were made up of leucocytes, granular detritus, of bacteria and eventually, of a remnant of epithelium and foreign bodies.

#### BODIES RESEMBLING PROTOZOA.

When the secretions and exudations of birds affected with croup contained remains of epithelium, the round, protozoa-like epithelial nuclei, referred to above, were present. Besides these, other very different bodies, which often more or less resembled the protozoa described as the cause of fowl diphtheria, or epithelioma contagiosum, were seen. No case of epithelioma contagiosum came under our observation. Table 1 shows some of the protozoa-like bodies found. These figures were drawn from fresh preparations which had been stained either with methylene blue, gentian violet, fuchsin, or Lugol's solution. The material was quite fresh, or had been stained without drying or heating. Many peculiarities could be distinguished with the methylene blue or Lugol's solution. The commonest forms of protozoa-like bodies were the swollen epithelial nuclei, which have been more minutely described above. They were

always most numerous on the outside of the cheese-like matter in nostrils, eyes, and bronchial tubes, as well as on the underside of the pseudo-membrane or in the material which was scraped from the mucous membrane. The protozoa-like bodies were present in the greatest numbers in material from the same source. If we compare the drawings of the different bodies in (Figs. 25), we find very many forms which agree with the protozoa found by Riva Silvestrini, Maggianti, Piana, Pfeiffer, Babès, Puscariu, and Galli-Vale

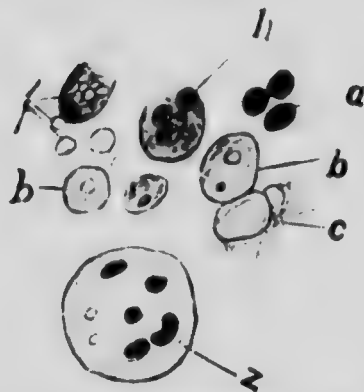


Fig. 14.—Protozoa-like bodies from the bronchial exudate of pigeon No. 1. *a* The nuclei of leucocytes. *b* Swollen epithelial nuclei with or without nucleoli. *f* Fat droplets. *A* Yeast cell with spores. *z* Probably a giant cell. Epithelial nuclei have a diameter of 8-10  $\mu$ , the other bodies are drawn on the same scale.



Fig. 15.—Bodies from the slimy purulent nasal secretion of pigeon No. 12. Same lettering as figure 14.

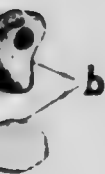
Round, amoeba and crescent-shaped forms, etc., were often present and contents of these bodies were homogeneous, granular, or partly homogeneous and partly granular. They stained either badly, or not at all. Very often, one or more nucleoli were present which appeared in unstained preparations as clear or reddish spots. In stained preparations, the one small nucleolus was often stained, while the other in the same cell had not taken the stain at all. Besides these central bodies, the protozoa-like forms often held enclosed large clear vacuoles, which could be stained black with osmic acid, and were, therefore, fat globules. Other similar globules as they appeared in pigeon 4. when 7 gave no fat reaction and might have been true protozoa, but were more probably yeast-cells. Such accidental existence of foreign bodies in the exudations is not a rare thing, as is shown by Figs. 18, 19, 22 and 23. Very many of the protozoa-like bodies were either enclosed in the remains of cells, or showed debris of cells that had been present.

Twice, motile protozoa were present. In the bronchial tubes of infected hen No. 41, there were some oval forms which moved by means of a wreath of short cilia around the head end. (*Infusorium diplocladum* (See Fig. 22, i).

On the surface of the firm exudations in the mouth of pigeon 6, round granular forms, each with three long cilia, were present. There is nothing remarkable in this, for motile protozoa were frequently found in the mouth cavity and intestines of perfectly healthy fowl.

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Kitt describes and gives drawings of protozoa, or "Molluscum-Koeper," as he describes them. They are irregular bodies which stain intensively, and were found in the cells of an epithelioma.

We observed the same bodies in the epithelial cells under the pseudo-membrane in the mouth of fowl 28, and they are shown in Fig. 5. Unstained, they were homogeneous, clear and greenish in color. They were stained dark blue with haemotoxylin. In those parts of the epithelium in which these bodies were absent, the cell nuclei were homogeneous, or finely granular, and very much swollen. The clinical form of this case of the disease was striking because the membrane always formed again with extraordinary obstinacy, and in some cases grew right into the bone tissue of the left lower jaw. Similar bodies were present in the exudation of bird 26, and inoculated fowl 29.

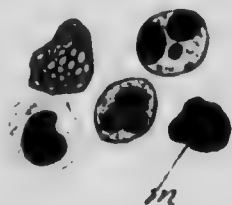


Fig. 16.—From the eyelid of fowl No. 29 structures resembling Kitt's "Molluscum bodies."

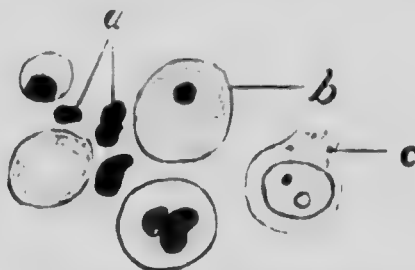


Fig. 17.—From the secretion and exudation from the eyelids of hens 135, 36 and 39. Swollen epithelial nuclei are 16  $\mu$  in diameter.

As appears from the drawings, the most varied protozoa-like bodies were present in the secretions and exudations, of naturally diseased fowls and of those inoculated with bacterial cultures.

In conclusion, we may state that most of the above described bodies were probably different products of degeneration of the epithelium and endothelium. Some must be regarded as foreign bodies and protozoa accidentally present in the exudations.

Kitt's molluscum bodies, in the epithelium of fowl 28, resemble the cell enclosures frequently found in Carcinoma, which are claimed by some as being the causal organism.

#### BACTERIA.

In all the pathological exudations and secretions, bacteria were present. Cultures in gelatine and agar plates and aerobic and anaerobic cultures in bouillon were usually made. In some cases, the material was only examined microscopically. In six cases of diseased fowls (29, 30, 33, 34, 35 and 36), *B. pyocyaneus* in pure cultures grew from spleen, liver, kidneys and blood. In all other cases, either some colonies of *B. coli* grew from cultures made from these organs, or the cultures remained sterile.

From the exudations and secretions, many different micro-organisms developed, and occasionally, yeasts and fungi were present. The colon bacillus

was sometimes isolated, and a large number of other bacteria-cocci, bacilli and a spirillum. The spirillum which had three or four turns could not be grown in any culture medium. Some five or six of the other micro-organisms were grown in pure-cultures, and their pathogenic properties were investigated by inoculations into guinea pigs, mice, and chickens. No results followed the inoculations; and, consequently, we must regard these forms as purely adventive.

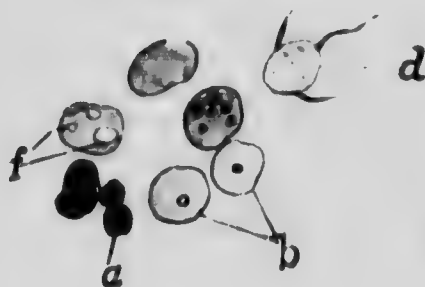


Fig. 18.—From the cheese-like exudate from the *cella infra-orbitalis* of pigeon 6. *d*. Is one of the Flagellata with three motile flagella.



Fig. 19.—From the underside of a diphtheritic membrane from hen No. 7. *p*. Probably some form of vegetable life.

We succeeded, however, in isolating two virulent forms of bacteria. The one was a typical *Bacillus pyocyaneus*. This bacillus was present in the cheese-like masses in the lungs of hen 9, in company with a few *B. coli*; in the firm exudations of the *Cella infra-orbitalis* of hen 32; in the cheesy masses of hens 20, 30, 31, 33, 34, 35, 36. In the six cases last mentioned, the bacillus was also isolated from the blood, liver, kidneys and spleen. Five of these birds were killed and had been dissected at once.

This *Bacillus pyocyaneus* was, as the inoculation experiments show, capable of producing typical croupous and diphtheritic membranes in mouth and eyes; it was the cause of severe tumors in the submucous, or subcutaneous tissue, the contents of which were firm, cheesy and yellowish-white. It produced purulent conjunctivitis; blindness; purulent panophthalmitis; inflammation of the lungs; and hard cheese-like exudations in the bronchial tubes. In a word, it produced symptoms identical with those of roup.

The second virulent form of bacteria isolated seems to be a new organism. We have named it *B. cacosmus* and shall also refer to it as the roup bacillus. It was in great numbers in the croupous membranes under the tongue of hens 1 and 9; in the cheesy material of the tumor on the head of hen 5, as well as in the purulent conjunctival secretion; in the nasal secretion of hen 4; in the purulent masses which closed the lacrymal duct of hen 21; and was also found in a number of other birds affected with roup.



Further, this bacillus was repeatedly isolated from the secretions of the hens and pigeons which had been inoculated with it. At first, it was usually present in the tumors of the submucous or subcutaneous tissue in pure culture. Later, it was found mixed with other bacterial forms in the tissues and also in the exudation products. In chronic cases of the disease, the roup bacillus could not be culturally detected, but it could often be identified microscopically in the cheese-like masses of diseased or inoculated fowls. We have never isolated this bacillus from the blood or other internal organs.

#### MORPHOLOGY OF *B. CACOSMUS*.

The roup bacillus is a small rod .25 to 5m.m.m. thick, .25 to 5m.m.m. long, with rounded ends. It often occurs in pairs and occasionally in short chains of 4 or 5 cells. The smallest forms are coccus-like, the longer are rods. With the ordinary aniline stains, they stain very well. In old bacilli, there are often only zones or parts of the cell that take the stain (involution forms). The bacteria are not stained by Gram's method. In young agar or bouillon cultures, the roup bacilli are very motile. They possess 4 or 5 peritrichous cilia which are three or four times as long as the bacilli. Capsules and spores are not formed.

#### CULTURES.

**Gelatine Plate Cultures.** Colonies appear in 24-28 hours as small, round, iridescent points, which grow slowly. They are flat, round, or somewhat irregular in shape. They never become prominent; but, as their growth continues,



Fig. 20.—From the nasal secretion of hen 11 and 26. Ciliated cell showing slimy degeneration.

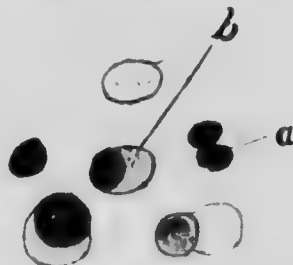


Fig. 21.—Bodies from a tumor from hen No. 17.

they sink deeper into the gelatine. The surface is smooth and shining. They are light grey in color. Through the microscope, young colonies are seen to be slightly granular. The centre is dark, yellowish or brown, the border zone clearer, and usually provided with darker, radiate markings; in older colonies, these disappear, and the gelatine becomes liquefied to the bottom of the plate. The growth at the edge of the colony lasts for about 8 days, and then ceases. In this time, they usually reach the size of from 3-5 mm., while after two days they were from 1-2 mm. in size. The submerged colonies appear as round, slightly granular, clear points, which quickly penetrate to the surface of the gelatine.

**Gelatine Streak Cultures.** Along the needle track, a thin, grey, shiny growth appears, under which the gelatine soon becomes furrowed. The growth sinks deeper into the gelatine, and the liquefied gelatine and the bacteria form as a turbid mass to the bottom of the tube.

**Gelatine Stick Cultures.** After 24-28 hours, a homogeneous, fine, grey band forms along the line of puncture. At the same time, on the surface of the gelatine, a smooth, fine covering grows, and, after two or three days, the gelatine becomes liquefied slowly around the puncture, spreads gradually over the whole surface of the gelatine, and then the liquefaction becomes stratiform. The liquefied gelatine is turbid, but neverropy. On the surface of the solid gelatine, a copious grey sediment settles. After two or three weeks, when about 2-3 of the gelatine has become liquefied, growth ceases. The liquefied mass gives a very strong alkaline reaction, and has a very disagreeable putrid smell.

**Agar Plate Cultures.** At 37 degrees C., in 24 hours the surface colonies appear as smooth, gleaming points, which grow quickly into round or irregular shaped masses. The centre becomes somewhat thicker and greyer. After two days, they are three to five mm. in diameter. The whole colony has an iridescent lustre. Deep colonies appear as grey points. Seen through the microscope, they are round, or somewhat irregular, clear, and darker at the margin.

**Agar Streak Cultures.** The culture spreads over the whole surface, and forms a smooth, faintly gleaming, thin, grey cover, which appears blue when the light falls on it. The condensation water becomes turbid and grey. (C)

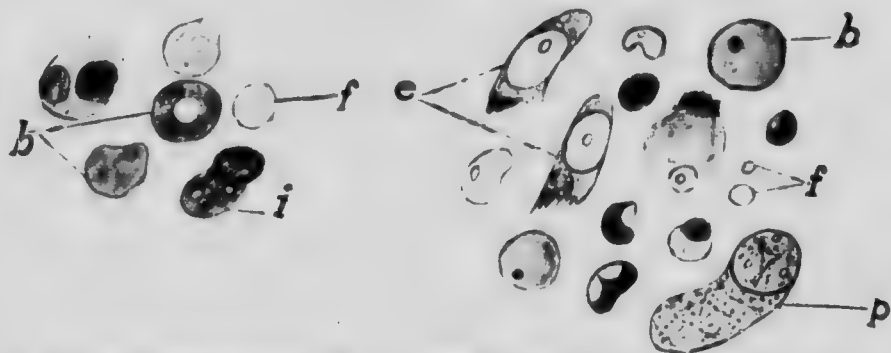


Fig. 22.—From the bronchial exudate No. 16. *b*. A motile infusorium with ciliated crown.

Fig. 23.—Bodies from the slimy pus from the *Regio olfactoria*, or hen No. 23. *c*. Ciliated cells with swollen nuclei. *p*. A foreign body, perhaps of vegetable origin.

agar, with 5 per cent. glycerine, the growth is the same, only much slower.

**Bouillon Cultures.** In 24 hours, the bouillon is uniformly turbid. The reaction is unchanged, and a grey sediment forms which diffuses uniformly when the test-tube is shaken. After 4 or 5 days, a thin, grey pellicle appears

on the surface, which is easily broken and then sinks in shreds to the bottom of the tube. The culture has a very disagreeable, sweetish odor. Old cultures are always very alkaline. Very old cultures become completely sedimented, and brown in color. Growth occurs in slightly alkaline or acid bouillon, and small lumps appear in the sediment.

**Milk Cultures.** In four days, the milk coagulates into a soft curd with a thin layer of turbid, yellowish-grey serum on the surface. The reaction is slightly alkaline, and the curd digests slowly, as it takes about four weeks for the curd to completely dissolve. The liquid is turbid, yellowish-grey in color, somewhat thick, and with an alkaline reaction. There is usually a thick, greyish-white sediment. The culture has a disagreeable odor.

On Potatoes, the rous bacillus develops fairly well, as a thin, grey, smooth and shiny layer.

In Dunham's solution, a uniform turbidity appears. At the bottom, a grey, flocky sediment forms. After three or four weeks the culture is dark brown, very alkaline, and has a putrid odor.

1 per cent. solution of Peptone with 4 per cent. Dextrose. In this medium, a copious, diffuse turbidity forms, as well as a granular sediment. 14 per cent. of gas collects in 48 hours. The reaction is slightly acid, and remains so.

**Temperature relations.** The rous bacillus grows best at a temperature of 37-40 degrees C., and it also grows well at room temperatures, 20-22 degrees C.

The thermal death-point (Sternberg's method) is 65 degrees C. for 10 minutes.

**Oxygen requirements.** It grows best aerobically. Anaerobic cultures are poor, and grow very slowly.

**Relation to sunlight and dessication.** An exposure of 2 hours to direct sunlight (Lat. 42) kills the bacillus exposed in agar dish cultures. It succumbs to dessication in 6 days.

**Action of disinfectants.** The disinfecting power of the following chemicals was :

|                                                                                            |                |
|--------------------------------------------------------------------------------------------|----------------|
| Corrosive sublimate, 1 per cent. ....                                                      | 10-15 seconds. |
| Lysol, 2 per cent. ....                                                                    | 1½-2 minutes.  |
| Carbolic acid, 5 per cent. ....                                                            | 12-15 seconds. |
| Creolin and glycerine, equal part. 5 c.c. in 100 c.c. water<br>(Friedberger-Froehner)..... | 1-2 minutes.   |
| Lime water (Sat. solution) ....                                                            | 10-15 minutes. |

#### VIRULENCE.

The virulence of the rous bacillus was at first weak, but was much increased by passing once or twice through pigeons. Like *B. pyocyaneus*, the rous bacillus produced suppuration, but the effect was strictly local.

**Pathogenesis.** Rabbits die after an intraperitoneal inoculation of 1 c.c.

of a young bouillon culture in from 18 to 24 hours. Dissection shows a wide spread, purulent peritonitis, with slight bloody extravasations of the peritoneum. Subcutaneous inoculations of small quantities of bouillon cultures lead to hot tumors, which spread quickly and in which firm, hard centres form. The whole tumor gradually assumes the same hard, uneven condition. The skin becomes dry, cracks, splits and forms a brown, thick crust which, after some time, falls off. Under the crust, there is an extensive, uneven yellowish-white, cheese-like mass, 2 mm. to 2 cm. thick, firmly adherent to the underlying tissue. Gradually this mass falls off, and regeneration of the tissue from the edges of the skin follows. The hard, cheese-like masses look exactly like the croup and diphtheritic membranes of fowls and pigeons. They consist



Fig. 24.—From the nasal secretion of hen No. 6. *m*, Kitt's "Molluscum bodies." *r*, A red blood cell.

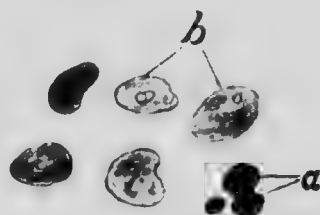


Fig. 25.—Bodies from the under side of a false membrane from pigeon No. 11.

of bacteria and leucocytes, as well as of some detritus. The general conditions of the rabbits is poor, so long as there is a hot tumor. Later, their condition improves, though the animals remain very thin in spite of being well fed. In a few cases, sympathetic inflammation of the eyes, purulent conjunctivitis on one or both sides, purulent panophthalmia, perforation of the cornea, etc., occur during the course of the healing of the local lesions. If there are no complications, the local lesions heal in from 3-5 weeks; if the described complications set in, death comes after the disease has run its course for 1-3 months.

Old cultures filtered through Chamberland filters, produced, when inoculated intraperitoneally, neither death nor sickness.

Guinea Pigs. The effect of the roup bacillus was the same on these animals as on the rabbits.

Mice die in 12 to 18 hours from inoculation with 1-8 to 1-4 c.c. of a bouillon culture of the bacillus. Dissection shows excessive swelling of the spleen. Intraperitoneal inoculation gives rise to hemorrhagic purulent peritonitis. The bacilli are present in great numbers in the exudations and spleen; whilst they are but seldom found in the blood.

The effect of the roup bacillus on hens and pigeons, is shown in the following table of inoculation experiments.

TABLE OF THE NATURAL CASES OF THE DISEASE.

| Number. | Slimy, putrid conjunctivitis with or without a soft swelling of the lids. | Pseudo membranes on the conjunctiva or cornea. | Phlegmonous swelling of the lids with hard putrid centre. | Diseases of the inner eye. | Submucous or subcutaneous tumors with solid putrid centre. | Slimy putrid catarrh of the nose and the secondary cavities of the nose. | Pseudo membranes in mouth and pharynx. | Pneumonia with or without exudations in the bronchial tubes, etc; | Affection of the pleura and peritoneum. | Catarrh and inflammation of the bowels. | Chronic course of the disease. Acute | Condition : Thin Good | Recovered Died Killed |
|---------|---------------------------------------------------------------------------|------------------------------------------------|-----------------------------------------------------------|----------------------------|------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------|-------------------------------------------------------------------|-----------------------------------------|-----------------------------------------|--------------------------------------|-----------------------|-----------------------|
| 1       | x                                                                         |                                                | x                                                         |                            | x                                                          | x                                                                        | x                                      | x                                                                 |                                         |                                         | x                                    | x                     | x                     |
| 2       | x                                                                         | x                                              |                                                           |                            |                                                            | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 3       |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 4       | x                                                                         |                                                | x                                                         | x                          |                                                            | x                                                                        |                                        |                                                                   | x                                       | x                                       | x                                    | x                     | x                     |
| 5       | x                                                                         |                                                | x                                                         |                            | x                                                          | x                                                                        | x                                      | x                                                                 |                                         |                                         | x                                    | x                     | x                     |
| 6       | x                                                                         |                                                | x                                                         |                            |                                                            | x                                                                        | x                                      | x                                                                 | x                                       |                                         | x                                    | x                     | x                     |
| 7       |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 8       |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 9       |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   | x                                       |                                         | x                                    | x                     | x                     |
| 10      |                                                                           |                                                |                                                           |                            | x                                                          | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 11      | x                                                                         | x                                              | x                                                         | x                          |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 12      | x                                                                         | x                                              |                                                           |                            |                                                            | x                                                                        | x                                      | x                                                                 | x                                       |                                         | x                                    | x                     | x                     |
| 13      | x                                                                         | x                                              |                                                           |                            |                                                            | x                                                                        | x                                      | x                                                                 | x                                       | x                                       | x                                    | x                     | x                     |
| 14      |                                                                           |                                                |                                                           |                            | x                                                          | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 15      | x                                                                         |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 16      | x                                                                         | x                                              | x                                                         |                            | x                                                          | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 17      | x                                                                         | x                                              | x                                                         |                            | x                                                          | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 18      |                                                                           |                                                |                                                           |                            |                                                            |                                                                          | x                                      | x                                                                 | x                                       | x                                       | x                                    | x                     | x                     |
| 19      |                                                                           |                                                |                                                           |                            |                                                            |                                                                          | x                                      | x                                                                 | x                                       |                                         | x                                    | x                     | x                     |
| 20      |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 21      | x                                                                         |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 22      |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 23      | x                                                                         |                                                | x                                                         |                            |                                                            | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 24      | x                                                                         | x                                              | x                                                         | x                          | x                                                          | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 25      | x                                                                         |                                                | x                                                         |                            |                                                            | x                                                                        |                                        |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 26      | x                                                                         |                                                | x                                                         |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 27      | x                                                                         |                                                | x                                                         |                            |                                                            |                                                                          | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 28      |                                                                           |                                                |                                                           |                            |                                                            |                                                                          | x                                      | x                                                                 |                                         |                                         | x                                    | x                     | x                     |
| 29      |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 30      | x                                                                         |                                                | x                                                         |                            |                                                            |                                                                          | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 31      |                                                                           |                                                |                                                           |                            |                                                            |                                                                          | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 32      | x                                                                         |                                                | x                                                         |                            | x                                                          | x                                                                        | x                                      | x                                                                 | x                                       |                                         | x                                    | x                     | x                     |
| 33      | x                                                                         |                                                | x                                                         | x                          |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 34      |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |
| 35      | x                                                                         |                                                | x                                                         | x                          |                                                            | x                                                                        | x                                      | x                                                                 |                                         |                                         | x                                    | x                     | x                     |
| 36      |                                                                           |                                                |                                                           |                            |                                                            | x                                                                        | x                                      |                                                                   |                                         |                                         | x                                    | x                     | x                     |

## EXPERIMENTS ON INFECTING CHICKENS AND PIGEONS.

Fowl Diphtheria or Roup, prevalent in America, appears and passes for an infectious disease, because we find that from twenty to ninety per cent. of all chickens in a flock may become diseased. It is, therefore, of great importance to know how infection takes place, and how the disease is transferred from bird to bird. In order to orient our observations with what has already been noted by others, it will be necessary to briefly summarize a portion of the literature which has appeared.

Zürn says that diphtheria is a highly infectious disease. He claims that secretion and exudation products of diseased birds are probable carriers of the poison, but he did not support this statement with experiments.

According to the statements of Siedamgrotsky, Friedberger and Perronci it is impossible, or very difficult, to infect healthy birds with the pathological products of sick ones. Cornevin and Nicati, on the other hand, inoculated the pathological secretions of diseased birds into healthy chickens and rabbits and produced diphtheria in these animals. According to Trinchard, it is easy to infect healthy birds, the time elapsing from the date of infection to the first symptoms of the disease being 7-20 days.

Babes and Puscaru were not able to produce the disease with diphtheritic membranes, containing bacteria and numerous trichomonas.

Massanti transferred the disease with fresh pseudo-membranes containing many flagellata. When he killed these protozoa with salt, he never succeeded in producing the disease. Loir and Ducloux infected healthy birds with the blood and any of the parts of organs from diseased ones. Kitt states that a transfer of croupous-diphtheritic membrane to healthy birds very frequently produces no infection. Friedberger and Froehner declare that diphtheria caused by protozoa can more easily be transferred to healthy fowls than diphtheria produced by bacteria.

Moore tried to infect healthy chickens with pathological secretions and exudations, and kept healthy fowls in with diseased ones; but in no case was he able to produce the disease.

In the following experiments, the diseased birds were obtained from the poultry department of the Ontario Agricultural College. Most of the healthy ones were purchased from different farms where no roup was present, but a few were secured from the Poultry Department where they had been exposed to infection, but had never become affected.

#### EXPERIMENTS TO ASCERTAIN IF HEALTHY BIRDS BECOME AFFECTED WHEN KEPT WITH DISEASED ONES.

Fowl No. 1. Small hen about 6 months old, in fair condition. To irritate the conjunctiva, a few drops of 2 per cent. acetic acid were dropped under the left eye-lid. Then the hen was placed in the cage with diseased chickens No. 1-5. 3 Dec. 01.

Dec. 9.—General conditions unchanged. From the left nostril a little serous fluid escapes, when one presses on the dorsal wall of the nose, more of this secretion exudes from the nostril.

Dec. 13.—The left nostril is closed entirely with dirty looking crusts of dried up secretion, mixed with food particles. Under this crust is a small quantity of a grey, sticky and putrid-stinking liquid.

Dec. 18.—Very copious stinking secretion runs from the left nostril. General condition disturbed. The bird generally stands at the back corner of the cage, in a crouching position, the head drawn close to the body and very often tucked under the wing. Eyes often kept shut, and the beak generally a little open.



Dec. 19.—Discharge from both nostrils. Nasal secretion is slimy and putrid.

Jan. 3, 1902.—Hen has lost very much flesh. All visible mucous membranes are pale. General condition occasionally normal, but generally greatly depressed. Appetite always fair. On the mucous membrane in the mouth is some sticky slime, and on both sides near the tongue there are two small greyish-white pseudo-membranes about 1 mm. thick. They can be easily removed. The mucous membrane under them is uneven, red and begins to bleed at various spots.

Jan. 8.—Pseudo-membranes have disappeared from the mouth. The left nostril is entirely closed by a dry, yellowish-white, cheesy mass, which can only be removed with difficulty, when the surface underneath bleeds. This diseased part was treated with a 2½ per cent. solution of Creolin.

Jan. 9.—At the place treated with Creolin yesterday, a new pseudo-membrane has formed. Treatment again renewed.

Jan. 11.—The left nostril again blocked up by the cheesy yellowish mass. In many places in the mouth, thin, grey pseudo-membranes and at two places on the left side of the mouth yellowish-white patches of croup-membranes have formed.

Jan. 14.—Both nostrils are free from any secretions, but the disagreeable smell continues. At several places in the mouth, small pseudo-membranes may be seen. General condition, fairly good.

From 15th of Jan. to 3rd of Feb.—Chronic catarrh of both nostrils, sometimes with serous, and at other times with much pus. From time to time, the discharge from the nostrils may stop for one or a few days. There is always a very bad smell present. Very often pseudo-membranes appear in the mouth, especially on both sides near the tongue, around the entrance into the larynx, or on the palate, etc., for one or a few days. They never grow larger than 1-4 cm. The general condition was always disturbed, sometimes more, sometimes less, with loss of flesh and anaemia.

Feb. 3.—Killed with chloroform.

Post-Mortem: Thin carcass.

Conjunctiva and mucous membrane of the mouth very pale, without pseudo-membranes. Nostrils are filled with dirty grey dried secretion. The mucous membrane in the regio olfactoria is very soft, red and swollen. It shows at several places, small, bloody extravasations. It is covered with a grey, slimy mass mingled with pus and bloody streaks. The lower parts of the nasal canals are blocked up with a mass, consisting of putrid slime, and solid granules of solid white pus. The whole mucous membrane and submucous tissues have disappeared. The cartilage of the left oral concha is at several places black, dry and necrotic, or grey with very bad smell. In the dorsal wall of the pharynx and the mucous membrane of the higher third of the oesophagus are very many lymphoid follicles, swollen and filled with a yellow cheesy matter. They extend often as deep as the muscles and consist of pus corpuscles and four different kinds of germs (short thick rods, single or generally two together; long chains of another germ; streptococci and staphylococci). The

organs are normal; spleen and liver small. Cultures from these two organs remain sterile. The putrid slime from the nostrils consists microscopically of slimy masses, desquamated epithelial cells, pus, erythrocytes and very many spirilla. A large coccus, several *B. coli* and *aerogenes* bacilli, and the rous bacillus in a few specimens, were isolated.

Fowl No. 2. Healthy cock, about one year old. 13th Dec. '01. Was kept in the same cage with fowl No. 1, and the diseased chickens 1-5.

Dec. 21.—General condition normal. At the right nostril, a small quantity of a clear serous fluid is visible.

Dec. 26.—The right nostril is entirely closed with thick grey, crusty secretion. On taking this away, large quantities of a grey, putrid, stinking slime exude from the nostril.

From Dec. 27 to January 10 there occurred chronic nasal catarrh; sometimes discharging and at other times stopping. Offensive smell from nose and mouth. General condition not much altered; appetite good. No pathological changes were observed on the mucous membranes in the eyes and mouth.

Jan. 29.—For eight days, the bird showed neither nasal catarrh nor any other abnormal appearance. Killed by decapitation.

Post-Mortem: Fairly well nourished carcass. In the nasal parts of the right ductus lacrimalis are some small, brown spots, encased in the mucosa.

Fowl No. 5. Large, strong, well-developed hen, about 1 year old. It was kept in a cage with infested fowl 4 for ten days.

Jan. 10.—Both nostrils are partly closed by a clear, serous slimy, fetid-smelling liquid. Mucous membranes very pale. General condition and appetite, normal.

Jan. 14.—At the entrance to the larynx, and on the dorsal wall of the larynx itself, a pseudo-membrane is located. It has an uneven surface, is yellowish-white in color, 1 to 2 mm. thick, and can be removed with little difficulty. The undersurface after removal begins to bleed at several spots.

Jan. 15.—At the place where the pseudo-membrane was removed, the mucous membrane is apparently normal. Discharge from the nostril almost entirely stopped.

From the 16th of January to the 4th of May chronic nasal catarrh of both nostrils, with fluctuating severity, occurred. Often the mucous membrane in the mouth had for one or several days small pseudo-membranes. General condition was abnormal during the later stages of the disease, with sleepiness, diminished appetite, and all visible mucous membrane pale. Chronic anaemia set in, as a secondary disease, following the rous.

Fowls Nos. 10 and 11 were healthy hens, about one year old. They were kept with the diseased chickens the second half of the month of December 1901, and the whole month of January, 1902. General condition remained unchanged. Eyes and nostrils remained normal. Several times on the mucous membranes in the mouth, small croupous membranes appeared for one or two days; then they disappeared again. The mucous membrane was pale, and was sometimes covered with a sticky slime.

Feb. 6th and 7th.—Left nostril of hen No. 10 shows a small quantity of serous, odorless fluid, which had disappeared the next day. These two hens remained normal and healthy.

Fowls No. 6, a., b., c., d., and e., were kept with the diseased chickens during the month of December, 1901, and January, 1902, without becoming infected. Later these chickens were used for experimenting with the roux bacillus, which had been made virulent by passage through pigeons. All reacted with typical symptoms of chicken diphtheria.

**EXPERIMENTS TO SHOW IF HEALTHY BIRDS COULD BE INFECTED WITH SECRETION OR EXUDATIONS OF DISEASED ONES.**

Fowl No. 4. Young, healthy hen. 17th Dec., 1901. Some of the sound, cheesy peritoneal exudation from diseased fowl No. 4, was rubbed on the sound or slightly scratched membranes (mucous) of both eyes, the mouth, and in the higher parts of the nostrils.

Jan. 18.—General condition normal. At the small scratch wound in the mouth the membrane is slightly swollen and red. A little clear, serous fluid exudes from the right nostril.

Jan. 19.—Mouth normal. Both nostrils are moist, with a little serous liquid having no smell.

Jan. 21.—The secretion from the nostril is grey, slimy and has a putrid smell. On the mucous membrane of the right side of the mouth, an irregular yellowish-white spot is located. It is about 1 m.m. thick and 1-2 cm. in size. This patch was taken off, and the membrane under it bled at a few spots.

Jan. 14.—Chronic, putrid nasal catarrh. General condition unchanged. Mucous membrane in the mouth always very pale, sometimes with small pseudo-membranes.

Jan. 21.—In the middle of the palate, a pseudo-membrane is located, about  $\frac{1}{4}$  cm. 2 in size, and 1 to 1½ mm. thick. It was removed and rubbed on the left conjunctiva.

Jan. 22.—The membrane has reformed in the mouth. Eyes are normal.

Jan. 23.—Some new membranes have formed at different places in the mouth.

From the 27th of Jan. till the middle of March, the chronic nasal catarrh disappeared, after having ceased several times for only one or a few days. The local infection in the mouth disappeared at the same time. General condition was good.

Fowl No. 5a. Healthy hen, half year old. On the 17th of December, some of the putrid nasal secretion from diseased chicken No. 4 was rubbed in and upon the mucous of the eye and the regio olfactoria.

This hen never became sick or diseased.

Fowl No. 7. A hen like No. 5a was inoculated on Jan. 3rd, 1902, with nasal secretion and pseudo-membranes from fowl 5. Inoculation on and under the mucous membrane of the mouth, and on the mucosa of the regio olfact. No visible                      on followed.

Fowl No. 9. This fowl suffered from chronic diarrhoea. It was kept in the stable for four months and then inoculated in eye-lids, mouth, and nostrils with croupous material from the eye-lid of diseased chicken No. 11.

Fowl No. 8. Healthy cock, three years old. Had been kept in the stable continually for three months.

Jan. 4.—On the scratched mucous membrane in the mouth near the tongue a croupous membrane from fowl 3 was rubbed in.

Jan. 5.—The mucous membrane at the place of infection was red, and covered with much saliva. General condition, normal.

Jan. 6.—At the place of infection, the mucous membrane is uneven grey; at several places, yellowish-white points appear.

Jan. 7.—Near the tongue, a croupous membrane has formed, yellowish-white in color, 1 mm. thick and about 1 cm. in area. The surrounding mucosa is reddened. After having taken off the membrane, the mucosa begins to bleed. At several other places in the mouth, very thin, greyish pseudo-membranes formed.

Jan. 8.—The pseudo-membrane near the tongue, which had been removed yesterday, has reformed, and is larger than before.

Jan. 15.—At the primary seat of infection, the croupous membranes have disappeared, but a small croupous patch is located on the left side of the mouth, and there are four others upon the mucous membrane of the palate, near the entrance to the nasal cavity.

From the 16th Jan. till the 25th Feb.—Often pseudo-membranes appeared on the mucous membranes in the mouth, and disappeared again in a few days. The general condition at this stage was poor. All visible mucous membranes were pale.

This fowl was killed and the post-mortem examination showed the existence of anaemia. No growths in cultures from the spleen.

Fowl No. 21. Young, healthy hen.

Jan. 3.—Was inoculated with slimy, putrid, nasal secretion from fowl No. 1, containing very many spirilla.  $1\frac{1}{2}$  c.c. was injected into the mucosa and submucosa of the mouth and nose,  $\frac{1}{2}$  c.c. into the right eye-lid and  $\frac{1}{2}$  c.c. into the right pleural cavity.

Jan. 4.—General condition unchanged. Eye-lid slightly swollen and conjunctiva moist. On the palate, one pseudo-membranous patch has formed,  $\frac{3}{4}$  c.m. in size, yellowish-white and cheesy. Mucous membrane of the mouth is pale.

Jan. 7, and later.—General and local condition normal.

Fowl No. 24a.—This bird was inoculated the 23rd of January, 1902, with material taken from the cheesy, degenerate lymph follicles in the oesophagus of fowl No. 1.

Fowl No. 25.—This chicken was inoculated with a solid mass of yellowish pus taken from the eye-lid of diseased fowl No. 21.

In both cases, the eye-lids became swollen for a few days. The mouth remained unchanged, and the general condition was never disturbed.

Besides these experiments, we tried to infect six other chickens (6-12

months old) with pathological secretions and exudation products. Several times the eye-lids became swollen for a few days, but no typical roup disease could be produced.

These results show that out of 10 healthy chickens, kept with diseased ones, five became infected. In four cases, nasal catarrh appeared in from 6 days to 1½ months. In the same birds, pseudo-membranes sometimes appeared in the mouth for a few days. In fowl No. 10 only, they appeared without any changes in the nostrils. Five fowl did not take the disease at all, although they were exposed to the same infection.

Of the 14 chickens and 1 pigeon (see later) which we attempted to infect with secretions and exudations from diseased fowls, only two fowls (Nos. 4 and 8) became diseased with roup. These experiments show that chicken diphtheria, or roup, may be transferred.

#### EXPERIMENTS TO INFECT HEALTHY FOWLS WITH *BACILLUS CACOSMIUS* (THE ROUP BACILLUS.)

Fowl No. 3. Small hen, weak constitution, about one year old.

Dec. 17.—The conjunctiva and the higher parts of the nasal mucosa were irritated with dilute lactic acid, after which a portion of a young agar culture of the roup germ (isolated from diseased hen 4) was rubbed in.

Dec. 18.—General condition unchanged. At the right nostril, some fluid can be seen.

Dec. 21.—At the right nostril, a small quantity of a slimy, putrid secretion is apparent. Disagreeable smell.

Jan. 8, 1902.—Typical chronic nasal catarrh is present. Mucosa of the mouth is pale. Under the tongue, an irregular croupous membrane is located, about 1 mm. thick. This membrane was difficult to remove, and after removal the surface beneath bled.

Jan. 9.—An extended new membrane has formed at the old place. It was again removed, and cultures were made from the mucous membrane lying beneath the false membrane, from which the roup germ was subsequently isolated.

Jan. 10 to Feb. 10.—Nasal catarrh remained chronic and putrid. The croupous membrane disappeared after having reappeared after four daily removals. Later, several small membranes appeared at different places in the mouth.

April 24.—The discharge from the nose has disappeared for 8 days, and there is no disagreeable smell. All visible mucous membranes are extraordinary pale. General condition, normal. No apparent loss of flesh during the disease.

Fowl No. 13. Healthy hen, about 8 months old.

Jan. 10, 1902.—½ c.c. of a bouillon culture, made from parts of the epithelial membrane under a removed croupous patch of diseased chicken 9, was subcutaneously inoculated. The culture was not pure, but the most numerous germ was the roup bacillus. At the place of inoculation, a hot tumor appeared, and became slowly resorbed in a few days.

Fowl No. 4. Weak hen, about  $\frac{1}{2}$  year old.

Jan. 12.—Inoculated in the mouth with an anaerobic bouillon culture, 4 hours old, made with the same material from diseased chicken No. 9. It contained coli and roup bacilli, and a streptococcus.

Jan. 13.—At the place of infection in the mouth, two small white have formed.

From 14th of Jan. till 10th of Feb.—General condition, normal. Several times, small croupous spots formed on the mucous membrane in the mouth for a few days.

Fowl No. 18. Healthy young hen.

Jan. 12.—Was inoculated with an aerobic culture from diseased hen No. 9 in the mouth and under the right conjunctiva. This culture contained the roup bacillus, and some long and thick rods, and a few cocci.

Jan. 14.—The infected eye-lid is slightly swollen. Mouth, normal.

Jan. 15th till Jan. 23rd.—General condition always normal. Swelling of the eye-lid disappeared. Mouth never affected.

Fowls 22 and 23. About 6-month-old hens.

Jan. 21.—A young agar culture of the roup bacillus, isolated from fowl 3, was inoculated in the mucous membranes of the mouth and nose without any abnormal changes subsequently taking place.

Fowl 26. Healthy hen, 1 year old.

Had suffered from natural roup at the beginning of winter, but wholly recovered after being diseased for two months.

March 15.— $\frac{1}{4}$  c.c. of a young culture of the roup bacillus, isolated from an infected pigeon, was inoculated in the left superior eye-lid, and in the pleural cavity.

March 16.—General condition very much disturbed. Eyes were kept shut, left eye-lid swollen, and warm. The conjunctiva is very watery, and grey. Under the eye-lid, there is much slimy matter. The bird breathed through the beak with loud inspiratory noise.

March 17.—General condition much improved. Respiration through the beak, swelling in the eye-lid diminished. Killed by decapitation.

Post-Mortem: Carcass in a fairly well nourished condition. Conjunctiva, pale and watery. In the depth of the submucous tissue of the left eye-lid, a solid mass of grey pus, about  $1\frac{1}{2}$  cm. long, is found. It can be extracted without breaking, being surrounded by a smooth brown membrane. At several places in the periorbital tissue, similar pus masses are present, but was sharply separated from the surrounding tissue. The interior eye is in tact. The nasal mucosa in the regio olfact, is highly reddened and covered with much slimy purid secretion. In the mucous of the left lacrymal canal are small bloody extravasations. Lungs are oedematous.

Fowl No. 27. Small healthy hen, about one year old.

Had several times been inoculated with the roup bacillus without result. On the 28th of April, this bird was again inoculated with a young agar culture of the roup bacillus, which had been passed through two pigeons.  $\frac{1}{4}$  c.c. was injected into each of the upper eye-lids, and 3 c.c. in the right pleural cavity.



The next day, the eye-lids were swollen, and the general condition of the fowl was disturbed. In a few days, all pathological symptoms had disappeared.

**Fowl 28.** Large strong hen, about  $1\frac{1}{2}$  years old.

Had been diseased with roup at the beginning of the winter, and entirely recovered.

**March 15.**—Was inoculated with  $1\frac{1}{2}$  c.c. of a 24-hour-old roup culture in the right pleural cavity, and  $\frac{1}{4}$  c.c. under the conjunctiva of the right eye.

**March 16.**—General condition disturbed. Small swelling of the eye-lid.

**March 17.**—Condition, normal.

**April 14.**—Subcutaneously inoculated with a 5 c.c. of a culture of the roup bacillus near the sternum.

**April 15.**—General condition, normal. Locally no change.

**April 22.**—5 c.c. of a 24-hour-old roup culture again subcutaneously inoculated.

**April 23.**—General condition much disturbed. The bird is apathetic and mopes in a corner of the cage. Locally, no change.

**May 1.**—General condition still disturbed, with loss of flesh. All mucous membranes are pale; no appetite.

The 14th of June, a stinking diarrhoea set in, and the fowl died on the 18th.

Post-mortem examination showed many grey, solid nodules in both lungs, and a slimy catarrh; at other places, a hemorrhagic inflammation of the thicker bowels.

**Fowl No. 29.**—Young hen which had been affected with roup in the early winter; healthy when inoculated.

**March 16.**—Inoculated in mouth and eye-lids with a culture of the roup germ, which had been passed through pigeons.

**March 17.**—Eye-lids considerably swollen, and hot. On the right side there is a serous secretion from the conjunctiva. Conjunctiva is grey, swollen and soft. General condition, highly disturbed.

**March 19.**—In the depth of the right upper eye-lid, a solid round tumor has formed. It is neither in direct contact with the mucosa or cuticle of the eye-lid, both being movable upon it. Conjunctiva, grey-brown in color. Slimy, putrid catarrh of the conjunctiva. The left upper eye-lid is but slightly swollen, and no conjunctival catarrh exists. General condition, very much disturbed. Bird apathetic, with almost no appetite.

**March 20.**—The tumor in the right eye-lid is better, and symptoms of acute inflammation have disappeared. Secretion of the conjunctiva is slimy and putrid. General condition, much better.

**March 21.**—Dead.

**Post-Mortem:** Carcass fairly well nourished. The solid tumor in the right upper eye-lid consists of a firm mass of pus, 2 cm. long,  $\frac{1}{2}$  cm. wide, and 2 mm. thick; color grey. It can be easily extracted, being surrounded by a dirty grey, smooth membrane. Conjunctiva is grey, and uneven. In the submucous tissue of the left upper eye-lid, there are a few grey layers of pus, not surrounded by a membrane, as in the right eye-lid. The inner eye and other organs unchanged.

Fowl No. 30. Healthy hen, about 2 years old, had been diseased with roup at the beginning of the winter; but apparently healthy when inoculated.

March 17.—Was inoculated with the solid matter (suspended in sterile water) from the eye-lid of fowl No. 26. A few drops were injected into the right upper eye-lid, 1 c.c. in the right thoracic cavity. The solid matter contained leucocytes and roup germs.

March 18.—Small swelling of the eyelid, without pathological secretion of the mucous membrane. General condition, very much disturbed.

March 20.—General condition, better. Sometimes the hen coughs. Swelling in the eye-lid mostly disappeared.

Later, the fowl recovered entirely.

Fowl No. 31. Had been diseased before. Inoculated in the same manner as fowl 30. After 24 hours, the general condition was very much disturbed, the eye-lid swollen, but the bird recovered in a few days.

Hen No. 32. Hen, gray, about one and a half years old. Had suffered with natural roup till New Year. Healthy now.

March 19.—Inoculated with a small quantity of the cheese-like matter from Pigeon 12 (which had been inoculated with the roup bacillus) on the mucous membrane of the mouth and in the pleural cavity.

March 20.—General condition disturbed. A croupous membrane has formed in the mouth, about  $\frac{1}{4}$  cm. in size and 1 mm. thick. It can easily be removed. The mucous membrane under it is uneven and grey.

March 20.—General condition, better. At several places in the mouth cavity, small pseudo-membranes have formed. Appetite lacking.

April 8.—Great loss of flesh. General condition, very bad. Fowl generally lies on the ground and does not care for food. Killed.

Post-Mortem: On the left side is a chronic indurative pleuritis (pleura thick and uneven, gray); liver small, spleen very large, without germs. The interior of the thin bowels contain a little food mixed with much slime. In the mucous membrane are petechial spots at many places.

Fowl No. 33.—Small hen, about 2 years old, had been diseased with roup. Was inoculated like fowl 32. Only a quickly passing disturbance of the general condition was the result.

Fowl No. 34.—Large, healthy hen that had never been diseased, about 12 years old.

March 24.—Inoculated with agar culture (28 hours old) isolated from pigeon 20 (second passage through pigeons). The culture was rubbed in the mucous membrane of the mouth, and 1-2 c.c. was injected under the mucous membrane of the upper eye-lids.

March 25.—General condition disturbed. Hen takes no interest in its surroundings, but cowers in a corner of the cage, with the neck drawn close to body, and eyes shut. The right, upper eye-lid is considerably swollen, and has its skin reddened. Conjunctiva gray. The left upper eye-lid is slightly swollen. Mucous membrane in the mouth is pale. Several yellowish-white pseudo-membranes are located on the tongue, and the mucous membrane near it. The submucous tissue on the bottom of the mouth cavity is oedematous.

March 20.—The oedema under the tongue has increased and spread over the face, up towards the eyes. The right eye-lids are sticking together, and cannot be opened. On the mucous membrane of the pharynx are several pseudo-membranous spots. The whole mucous membrane in the mouth is covered with clots of saliva.

April 2.—General condition improved. The right eye is always kept shut, and the lids are sticking together. Beneath the eye-lid there are putrid, slimy masses, and conjunctiva is dirty gray. The upper eye-lid is much swollen, the swelling having extended especially towards the nostrils.

April 8.—Condition the same. On the conjunctiva of the right upper eye-lid, a croupous membrane is located, yellowish-white, uneven, 2-3 mm. thick, and about  $\frac{1}{4}$  cm. in size. It can be removed. The mucous under it is uneven, gray and begins to bleed at several places. The oedema under the tongue has become absorbed. A small pseudo-membrane has formed on the palate near the entrance to the nasal cavity.

April 10.—General condition very bad. The fowl cowers in a corner of the cage, and pays no attention to its surroundings. It keeps its eyes shut, and the feathers are in great disorder. The appetite is very poor, and the whole face is much swollen. The wattles are swollen and prominent.

April 13.—General condition is improved. The purulent secretion from the right eye has diminished. The swelling of the face still exists. At several places fluctuation can be noticed; at others, an emphysema.

April 16.—The whole tumor is less hot, and is in an emphysematous condition. The tumors in both upper eye-lids are in communication with the tumor of the whole face. A croupous membrane still exists on the palate.

April 24.—General condition very bad. The hen lies most of the time on the floor of the cage in a comatose condition. The local changes have remained as they were, but the purulent conjunctivitis on the right side has almost entirely disappeared.

April 26.—Dead.

Post-Mortem: Carcass almost destitute of flesh and fat. Mouth and nares seemingly normal. Spleen a little swollen. In the subcutaneous tissue of the head there is a putrid abscess, the pus is dark brown, liquid, mixed with solid cheesy masses, and stinks offensively. The walls of the abscess are formed by a pyogenous membrane, which is covered with a solid layer of cheese-like, dry matter, having the appearance of the pseudo-membranes in the mouth. The croup bacillus was found in the pus, together with large numbers of coli-like bacteria. The spleen contained some coli-like bacilli.

Fowl No. 35.—Healthy hen, about one year old, which had never had roup. It was inoculated with the same culture as fowl No. 34.—two drops in the upper eye-lid of the right eye, and two drops into the high parts of the nose, the needle of the syringe being thrust through the dorsal wall of the nose.

March 24.—General condition much disturbed. Swelling of the right eye considerable, hot and firm, and the skin reddened. In the eye-lid, large quantities of slimy lacrymal secretions are present. Conjunctiva gray and swollen. Two small pseudo-membranes are located on the Conjunctiva of the upper lid.

The right nostril is covered with dirty gray, partly dried secretion. Under tongue a croupous membrane has formed, being about  $\frac{1}{4}$  cm. in size.

March 26.—General condition bad. The hen lies on the bottom of cage in somnolent state; occasionally it wakes up, rises and walks around, taking some food or water. The tumor in the right eye-lid has grown down to the face as far as the wattles. It is soft and hot. The eye-lids are sticking together. By opening them, a large quantity of a purulent secretion exudes; a clear jelly-like mass about one c.c.m. in size, can be removed from the deep of the eye-lid. A pseudo-membrane, about 2-3 mm. thick, has formed on the conjunctiva at the bulbus oculi, just over the cornea. On removal, the mucous membrane beneath begins to bleed. The membrana nicticans is greatly swollen. On the interior side a pseudo-membrane has formed about 4 mm. in size. The pseudo-membrane in the mouth has increased in thickness and size. Purulent secretion exudes from the right nostril.

March 27.—General condition, better. The membrane on the bulbus oculi has reformed and the jelly-like mass in the lid sack has reappeared, but is now turbid and gray.

March 28.—The secretion from the nostrils smells offensively. The right eye-lids are again sticking together, and the eye-lid contains a jelly-like, grayish mass.

March 29.—General condition is very bad. The eye-lids are sticking together and bulge out, because a yellowish-white, solid cheesy mass fills the whole eye-lid. This mass has formed from the jelly-like mass, which had been removed yesterday.

April 1.—Dead.

Post-Mortem: Fairly well nourished carcass. Left eye normal. The right eye-lid diffusely swollen. Between the partly opened lids a cheesy tumor protrudes (See Fig. N. 11). It fills the whole eye-lid and can be removed by pressing on the eye-lids. The conjunctiva gray and uneven. On the upper eye-lid a small pseudo-membrane has formed; similar membranes are located on the outside and inside of left eye-lid. Small masses of solid yellowish matter can be found in the subcutaneous tissue of the upper and lower eye-lid. The nasal cavities are blocked up with cheesy yellowish-white matter. The right infra-orbital cavity is filled up with the same cheese-like substance. On account of the pressure of this mass, the lateral bone-wall has disappeared and the cheesy mass lies directly under the skin of the face. The mucosa of the nares is grey and very soft. The mouth contains sticky masses of saliva. Spleen and liver are small and normal. Cultures made from portions of these organs remained sterile.

Fowl No. 36. Young healthy hen.

March 24.—Was inoculated like fowl No. 35, in the nose and right eye.

March 25.—General condition is considerably disturbed. Eye-lids swollen with conjunctivitis, on the upper lid is a small croupous membrane. Nostrils are dirty. A small quantity of yellowish-white liquid can be pressed out of the right one.

March 26.—The pseudo-membrane on the eye-lid has spread upon the bulbus

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oculi. It is about 1-2 mm. thick, and was removed. The mucous membrane under it is grey and uneven. Several small thin membranes have appeared near the tongue.

March 27.—The membrane in the eye has reformed. General condition improved.

April 2.—A solid, smooth tumor has formed in the depth of the right upper eye-lid. It has extended towards the nostrils.

April. Chronic, putrid conjunctivitis. Eye-lids often sticking together. Croupous membranes of the Conjunctiva always reappear after removal. Small pseudo-membranes often appear in the mouth. General condition disturbed with loss of flesh. Killed.

Post-Mortem: Eye-lid contained gray, slimy masses. Mucous is uneven. A cheesy membrane, about one cm.2 in size and two to four mm. thick covers the mucosa of the upper eye-lid. (Fig. 9). It adheres firmly to the mucosa, and is directly connected with solid, cheesy matter in the depths of the eye-lid. The mucous membrane of the nares shows a few small, bloody extravasations. Other organs are normal.

Fowl No. 37. Healthy, young hen which had had roup in the early winter. Inoculated like Fowl No. 36 in the left eye. After 24 hours, the eye-lid was swollen. Later a heavy, putrid catarrh of the conjunctiva set in, and pseudo-membranes formed several times on the conjunctiva. The general condition was more or less disturbed, and the appetite normal.

April 11.—The posterior half of the cornea was covered with an irregular pseudo-membrane, about 1½ mm. thick, yellowish-white, the other half clear, with the exception of a small turbid border near the pseudo-membrane. At the same time, the conjunctival secretion smelt offensively.

April 12.—A great part of the pseudo-membrane had disappeared, leaving the cornea uneven and gray. Two small membranes have formed on the upper eye-lid. At the place where the cornea had been covered by the pseudo-membrane, an ulcer developed, grew deeper and deeper, and opened on the 17th of April into the anterior eye cavity. The lens fell prolapsed and grew together with the perforated cornea. The cornea wound soon became filled up with a gray granulated tumor, which regenerated slowly.

The general condition was very much disturbed, and never again became normal.

On the 1st of May, the general condition was very bad. All visible mucous membranes were very pale. The swelling in the eye-lids and the conjunctival catarrh have disappeared, but the hen was blind. Death occurred on the 4th of June.

Post-Mortem: The Post-mortem examination showed all symptoms of severe anaemia. The corpora vitrea were partly softened, the retina split in many places and portions of the retina were mixed with the liquefied corpus vitreum.

Fowl No. 38. Hen about two years old.

April 3.—Was inoculated with virulent Roup bacillus, 1-5 c.c. under the conjunctiva of the right eye, and on the scratched mucous membrane of the mouth.

April 4.—The eye-lids were inflamed, hot and sticking together. Under the eye-lid, there is much slimy lacrymal secretion, and a jelly-like mass. The places of inoculation in the mouth are covered with thin pseudo-membranes. General condition disturbed. Later on, several small croupous membranes formed on the mucous membrane in the mouth.

April 11.—The whole eye-lid was filled with a cheesy, solid mass, which could easily be removed. Conjunctiva dirty gray. On the upper eye-lid a fairly large pseudo-membrane has formed. The next day the cheesy mass had reformed larger than before.

April 24.—A large solid tumor has formed in the depth of the upper eye-lid. The pseudo-membrane could scarcely be removed. Later, the putrid conjunctival catarrh ceased, the pseudo-membrane disappeared, the tumor on the eye-lid remained unchanged. The general condition was slightly disturbed, and the hen lost flesh.

Fowl No. 39. Inoculated with a Roup culture, which had been passed twice through pigeons, but then cultivated on agar for three weeks. The eye-lids became swollen, but the swelling soon subsided. General condition remained normal. The culture, therefore, had lost most of its virulence.

Fowls No. 42-45. Healthy young hens, inoculated with freshly isolated virulent Roup bacillus, on the mucous membrane in the mouth. Everywhere croupous membranes appeared at the places of inoculation. There they remained for a few days, and generally reappeared after removing once or twice.

The most severe reaction occurred in fowl 43 the day following the infection; the general condition was very much disturbed, the beak was kept open and much saliva was found in the mouth. The palate was covered with a whitish croupous membrane. Other membranes were located in the pharynx.

The last pseudo-membranes disappeared in all these infected fowls in two or three weeks.

#### PIGEON INOCULATIONS.

Pigeon No. 1. This bird was inoculated in both eye-lids, and on the mucous membrane in the mouth, with cheesy matter taken from the wattles of diseased fowl No. 24.

Feb. 18, 1902.—The only result was a swelling of the eye-lids, which quickly disappeared.

Feb. 27.—Condition normal. Some croupous membrane taken from a diseased fowl was rubbed on the mucous membrane of the pigeon's mouth and on the conjunctiva.

March 4.—Normal. Inoculated  $\frac{1}{4}$  c.c. of a culture of the roup bacillus which had been grown for several months on agar. The next day the bird was weak and somnolent, with no appetite. Both eyes are kept shut, the eye-lids sticking together; conjunctivitis.

April 17.—The acute conjunctivitis generally became chronic and putrid but disappeared after a while. No pseudo-membranes formed on the conjunctiva, but several of them appeared on the mucous membrane of the mouth for several times. The general condition was better.



Inoculated 2 drops of a roup culture (24 hours old) in the left conjunctiva on the upper eye-lid, and into the mucous membrane of the mouth.

April 18.—General condition very much disturbed; eye-lids sticking together, and swollen. The lid-sack contains slimy tears. A whitish pseudo-membrane has formed on the palate. Inoculated 3 c.c. of a filtered (sterile) bouillon culture under the skin.

April 19.—Condition like yesterday. The filtered culture has been entirely absorbed.

April 28.—The chronic catarrh of the conjunctiva still exists. In the depth of the upper eye-lid, a solid tumor has formed. Good general condition.

Pigeon No. 4. This bird was inoculated on the 18th of February with cheesy masses, taken from the lungs of fowl 41 (*B. pyocyaneus*), on and in the mucous membrane of the eye and mouth.

Feb. 25.—General condition, not disturbed. The day following the infection, small croupous membranes formed at several places in the mouth. The whole palate is covered with a yellowish-white diphtheritic membrane which was difficult to remove; the mucous membrane was uneven, gray and bleeds. The eye-lids are slightly swollen.

March 6.—The pigeon is apparently normal.  $\frac{1}{2}$  c.c. pure culture of the roup bacillus was inoculated into the left pleural cavity.

March 8.—General condition very badly disturbed.

March 13.—General condition has slowly improved. All mucous membranes are very pale. On the palate, a small croupous membrane has formed. Killed.

Post-Mortem: Left lung is firmly adherent to the pleura. The central part of the lung, that is, the region of the branching off of the larger bronchial tubes from the wind-pipe are solid, and inflamed. In the centre of this inflamed area is a solid, dry cheese-like, yellowish-white mass, measuring about 1 c.c. in diameter. This mass is partly surrounded with a smooth red-brown membrane. Spleen is small, and cultures made from it remained sterile.

Pigeon No. 3. Inoculated the 18th of February in the same manner as pigeon 4, and in addition 1 c.c. of the cheesy mass, triturated in sterile water, was injected in the left pleural cavity. The general condition was very much disturbed for the first 3 days after inoculation. The eye-lids were swollen, and a few small croupous membranes appeared in the mouth.

Feb. 27.—Condition normal. Some of the mucous membrane from fowl No. 27 was inoculated, after trituration in sterile water, into the mucous membrane of the eyes and mouth. No reaction followed.

March 6.—1-5 c.c. of a culture of the roup bacillus was injected into the left upper eye-lid. The eye-lid became very much swollen, and an acute serous conjunctivitis developed, and became slimy and putrid. A solid tumor formed in the swollen eye-lid. The general condition was much disturbed at the beginning and slowly became better.

May 6.—General condition normal. Local conditions have disappeared with the exception of a solid tumor in the upper eye-lid.

Pigeons No. 5, 6, 7, 10, and 11. These birds were inoculated in the mouth

and eye-lids with about 1-5 c.c. of a bouillon culture of the rous bacillus, which had been growing on artificial media for about two months. The only results were slight swelling, which generally disappeared after a few days. They were inoculated again with the rous bacillus, which had been previously passed through two pigeons. Local swelling followed the injection, with putrid chronic catarrh of the conjunctiva, and diphtheritic membranes in the mouth.

In pigeon No. 5, on the 20th day after the infection, a bad swelling, with putrid catarrh of the nostrils, set in, and remained until death occurred. The tumor in the left upper eye-lid grew down towards the nostrils. The side of the face became very much swollen, hot and painful. After a while, the center of the tumor became solid, and the left half of the palate began to grow down into the mouth cavity. At the lateral side of the palate, croupous membranes appeared, and always reappeared after removal. At other places in the mouth and pharynx, similar membranes appeared. The general condition of the bird was always disturbed. It lost most of its flesh, and died the 14th of April. (Second inoculation had taken place on the 9th of February).

Post-Mortem: A putrid chronic conjunctivitis is present. The tumor on the left side of the face is formed by a solid yellowish-white cheesy mass, lying just under the skin. This cheesy mass protrudes into the infra-orbital cavity, and also fills the nasal cavities. The tumor had pressed down the lower part of the palate towards the mouth cavity and caused a total absorption (by pressure) of the bone at the lateral edge. Here, a thick yellowish cheesy-like pseudo-membrane had formed, and this was in direct contact with the cheesy matter in the infra-orbital cavity. The lower parts of the nasal cavity contained a little putrid, slimy secretion, which had an offensive smell.

In Pigeon 6, a putrid catarrh of the nares appeared the 6th day after the second infection (19th of March). The left palate began to be pressed down towards the mouth. On the 11th day (24th of March) both nostrils were entirely closed with dried secretions, and the bird kept its beak open for breathing. The left eye was shut, the lids sticking together. On opening the lids, a putrid, slimy secretion, containing many solid, cheesy masses, could be pressed out. The anterior part of the cornea was covered with yellowish, uneven pseudo-membranes. This was removed, and the remaining cornea had the appearance of a cancer. It was soon covered again with a pseudo-membrane.

The general condition had not been very much disturbed at the beginning, but grew worse towards death, which occurred the 27th of March, that is 15 days after the second, or 39 days after the first inoculation. (See Fig. 8).

Post-Mortem: Carcass in poor condition. Nostrils and left eye are covered with dirty, offensive smelling secretions. The left conjunctival sac contains a slimy, putrid, lacrymal secretion, and a solid, cheesy mass. The conjunctiva is gray and uneven. Only a small part of the cornea, the posterior edge, has a normal appearance, the other part being covered with a pseudo-membrane, which had spread upon the surrounding conjunctiva. No turbid zone between diseased and healthy cornea exists. Under the pseudo-membrane, the cornea has the appearance of a cancer. A solid yellowish, cheesy mass was extracted from the left upper eye-lid. There was also a little tumor

under the lower eye-lid. The infra-orbital cavity is filled with cheesy matter. The palate is pressed down towards the mouth cavity. The mucous membrane of the nares is gray and swollen. The nasal cavities are filled up with a stinking, putrid, slimy secretion. A small pseudo-membrane is located in the pharyngeal membrane. Liver is enlarged; spleen small, and contains a few coli-like bacteria.

In Pigeon No. 7 no nasal catarrh set in. The putrid secretion from the left eye smelt offensively. Conjunctival catarrh and swelling of the eye-lids had disappeared in six weeks. Inoculated a third quantity of a virulent culture, with the same severe reaction as with the second.

Pigeon No. 10 suffered from a severe putrid catarrh of the conjunctiva for two months after the inoculation. In the latter stage of the disease, severe diarrhoea set in.

Pigeon No 11 had been inoculated the second time with 1 c.c. in the pleural cavity, and with a few drops in the right upper eye-lid. General condition was very much disturbed at the beginning; later, it became better, only to become worse again. A putrid catarrh of the right conjunctiva developed, and a solid tumor formed in the depth of the upper lid. 7 days after the second infection the pigeon was dead. (23rd of March).

**Post-Mortem:** From the eye-lid, much solid, cheesy matter was extracted. The conjunctiva was gray and swollen; the cornea turbid, but smooth; the corpus vitreum was partly liquefied, turbid, and contained pieces of the destroyed retina; the periorbital tissue has a few gray spots of pus. The central part of the right lung is completely congested, red, and contains a small, putrid centre. The pus was liquid, with an offensive smell.

Pigeon No. 15. This was similar to that of Pigeon No. 11. The bird died 5 days after the inoculation.

Pigeon No. 9. Was inoculated with a virulent culture of the rous bacillus by rubbing this on the mucous membranes of the mouth and nostrils. (5th of March).

Small croupous membranes appeared in the mouth. General condition was much changed. Five days later the pigeon was killed.

**Post-Mortem:** The lower parts of the nasal cavity contained much putrid secretion. The left infra-orbital cell was filled up with a solid, cheesy mass. The mucosa of the nose was gray, with small blood extravasations at several places; a cheesy mass had formed, in the submucous tissue underneath the tongue. Near this place, the mucous membrane bears a small diphtheritic spot.

Pigeon No. 12. Was inoculated 14th of March with a rous culture (freshly isolated from the nares of fowl No. 23), in the mouth, nose and pleural cavity. General condition was a little disturbed the next day, when a croupous membrane formed between larynx and base of the tongue. Later on, other pseudo-membranes appeared.

March 18.—Near the left angle of the beak, a new croupous membrane has formed, and the left nostril was partly blocked up, with an offensive, gray secretion. General condition bad. The bird lies most of the time on the bottom of the cage in a somnolent condition.

March 19.—Dead.

Post-Mortem: The central part of the right lung was inflamed. The lung contained two, small pneumonic spots. All other interior organs were normal, the spleen containing but a few colon bacilli. The nasal cavities were filled up with a slimy, gray offensive secretion, which contained many cheesy masses. The left infra-orbital cavity was full of a yellow, cheesy matter which was in direct contact with an extensive pseudo-membrane on the palatal and left side of the mouth cavity.

Pigeon No. 13. Was inoculated with rous bacillus like pigeon No. 12. The general condition was disturbed. Six days after the infection, the pigeon was killed.

Post-Mortem: Nares apparently normal. Under the tongue, 2 small pseudo-membranes have formed. The submucous tissue contains a solid cheesy mass, about as large as a hazel nut.

Pigeon No. 14. Was inoculated with a rous culture which had been growing in bouillon for 4 weeks. 1-5 c.c. was injected into the conjunctiva of the upper eye-lid and mouth. The general condition became disturbed. The eye-lids became swollen, and a solid tumor formed in it. This tumor began to get soft again after two months, and finally showed typical fluctuation. Size of the tumor, about 2 c.m.2. Two and a half months after the infection (14th of April), the pigeon was killed.

Post-Mortem: The tumor in the eye was caused by an abscess. The centre contained liquefied, putrid, gray pus. The walls were formed by a solid cheesy matter, which again was surrounded by a pyogenous membrane.

Pigeon No. 16. Was similar to pigeon No. 14.

Pigeon No. 23. Was inoculated with a virulent rous culture. The general condition was considerably disturbed. Small croupous membranes formed on the mucous membrane in the mouth. Five days after the infection (14th of April), a serous catarrh of the left nostril became apparent. Two days later, the pigeon had a very offensive smell. These symptoms disappeared after a few days, and the pigeon recovered.

#### RESUME OF INOCULATION EXPERIMENTS.

Reviewing our experiments with the 24 chickens, we see that to produce a typical case of rous, it was necessary to use a freshly isolated rous bacillus culture (fowl No. 3). Fowls 22 and 23 had been inoculated with the same culture grown for some time on artificial media. This caused considerable loss of virulence and failed to produce the disease.

Fowls 13, 17 and 18 were inoculated with young cultures from some of the diseased parts of naturally infected fowls. These were not pure rous cultures, and their effect upon the chickens was only a slight one. By passage through pigeons, however, the rous bacillus was made more virulent. The bacillus was usually isolated on agar or gelatine plate cultures.

The fowls 26, 28-33 were infected with a culture passed through one pigeon. These fowls had been suffering from natural rous in early winter, but at the

time of their inoculation were quite healthy. Four out of the six became diseased, while the rest showed only passing symptoms and disturbances.

A second series of experiments was carried on with roup cultures which had been passed through two pigeons. The chickens concerned in these experiments are No. 34-39 and 42-45. One of them, i.e., fowl 39, was infected with a culture which had been grown on artificial media for three weeks, after isolation from the second pigeon; in this case the bird was only very slightly affected. In all the other fowls, the cultures were used as soon as possible after isolation from the last pigeon. All reacted with pronounced symptoms comparable to those of a natural attack of roup. Two cases ended in death in five and thirty-three days respectively. Two lead to chronic loss of flesh and were anaemic. Fowl 36 was killed after a bad attack which lasted 22 days. The other diseased fowls recovered in 3 or 4 weeks.

The virulence of the roup bacillus might be made even more virulent by passage through a longer series of pigeons.

The pigeons themselves can be infected with the roup bacillus and show symptoms identical with those in chickens, but we seldom hear of pigeons suffering from roup under natural conditions, but they may become diseased as has been proven by these experiments. Very probably, they are more resistant against the natural channels of infection, being kept in relatively healthier localities than the average flock of farm chickens.

Fowl No. 27 could not be infected even with large quantities of the virulent cultures of the roup bacillus. It seemed to possess a natural immunity to roup.

Subcutaneous injections of 1-8 c.c. to  $\frac{1}{4}$  c.c. cultures of the roup bacillus caused only slight disturbances of the general condition. The skin was often colored green about 24 hours after the injection, and occasionally small solid tumors (solid pus) formed. Larger quantities (2-5 c.c.) of the culture caused large tumors, which were hard and painful at the beginning; later, a hard solid, smooth or uneven, tumor formed in the depth (similar to the effect on the eye-lids), when the symptoms of acute inflammation disappeared, the solid tumor remained as a foreign body for months; and the fowl lost flesh, became thinner and thinner, anaemic, and often died in from two to six months after inoculation, or recovered very slowly. The solid tumors are nothing but a mass of solid cheesy pus (see fowl 40 and pigeon 5).

Injected into the muscles, the roup bacillus causes extended necrosis of the tissue, which smelt offensively.

In the pleura and peritoneum, putrid inflammations are produced, from which the fowls may recover, but often die after a chronic disease (anaemia) lasting from 2 to 12 weeks.

Two chickens and two pigeons were fed with cultures of the roup bacillus mixed with their food, and no bad results followed.

Fowls and pigeons once infected with roup could be infected a second time after recovery from the first attack. Birds which had suffered from a natural attack of the disease and then recovered, also became again infected by the inoculation of the Roup bacillus; and finally serum from naturally or artificially

infected birds did not show any apparent influence on the roup bacillus. Test tubes, traces of an agglutination appeared in proportions of 1:50 or 1:100. The bacillus does not form a strong toxin. Old cultures filtered through porcelain inoculated in doses of 3-6 c.c. produced no local or general disturbance.

Bouillon cultures heated to 65 degrees c. for 10 minutes and inoculated in doses of 1-4 to 1 c.c. into the eye-lids of healthy fowls produced considerable inflammation, which disappeared in 2 days. 2-5 c.c. inoculated into the pleural or peritoneal cavity disturbed the general condition for 1 to 3 days, and produced trembling of the muscles.

We attempted to immunise several fowls and rabbits with the roup bacillus first injecting small quantities of heat killed cultures, followed by small doses of living cultures. The amount inoculated was slowly increased, but as soon as we began to use large amounts, small tumors formed beneath the skin. These became solid, hard, and had no tendency to become absorbed or to produce abscesses. They remained under the skin and led to chronic anaemia and death of the animal. After intraperitoneal injection of the cultures, the animals became chronically diseased, and grew thin and anaemic. The rabbits very often suffered with a secondary, putrid inflammation of the eyes.

These attempts to produce immunity were finally abandoned, as it seemed impossible to produce immunity by the methods described above.

#### EXPERIMENTS TO INFECT HEALTHY FOWLS WITH BACILLUS PYOCYANEUS.

Fowl No. 41. Healthy hen, about 1 year old.

Jan. 12.—Was inoculated with a 24-hour-old culture of *B. pyocyaneus* which had been isolated from diseased chicken No. 19, by rubbing a little of the culture upon the mucous membrane of the eyes, and injection of  $1\frac{1}{2}$  c.c. into the pleural cavity.

Jan. 13.—General condition considerably disturbed. There is hot swelling of the left eye-lids, serous conjunctivitis. Conjunctiva is gray, swollen, and at several places covered with croupous membranes, which are easily removed.

Jan. 26.—General condition more or less disturbed; no appetite; putrid conjunctivitis of the left eye; the cornea is turbid, gray, and uneven; the beak is generally kept open for breathing. Mucous membrane is gray and covered with much slimy saliva.

Feb. 17.—General condition is very much disturbed. The fowl is almost a skeleton, and lies in a corner of its cage in a somnolent condition. The respiration, especially the inspiration is very irregular. Mucous membrane and crest are cyanotic. A chronic, putrid conjunctivitis exists on the left side. Cornea gray; right eye seems to be normal, but the fowl is unable to see. Killed.

P. Mortem: Left cornea is uneven, gray, and covered with a thin putrid mass. The corpus vitreum is turbid, partly liquefied, and mixed with parts of the destroyed retina. A solid mass of cheesy matter has formed in the pleural membrane between heart and lungs, and the surrounding membrane.



is changed into a thick mass (2-3 mm.) of granulation tissue of a brown color. On the left side, all bronchial tubes in the region of the larger branches of the wind-pipe are filled with solid cheesy matter. The lungs themselves contain a few small pneumonic spots, which contain cheesy centres. The *B. Pyocyaneus* was isolated from all diseased parts. The cheesy mass itself contains nuclei of epithelial cells and a few real protozoa, motile, with short cilia (see Fig. 21.).

Fowl No. 41a. Was inoculated on the mucous membrane of the mouth and nose with the same culture as fowl 41. The next day (February 13th), the mucous membrane in the mouth was reddened, and showed a few blood-extravasations. These symptoms soon disappeared.

Fowl No. 46. Healthy, gray hen.

April 18.—Was inoculated with 1-5 c.c. of a young culture of *B. pyocyaneus* under the conjunctiva of the left upper eye-lid, and on the scratched mucous membrane of the mouth.

April 19.—General condition unchanged. Eye-lids hot, swollen, and sticking together. Conjunctival sack contains a slimy, putrid secretion. Mucous membrane in the mouth is gray and covered with slimy saliva.

April 21.—On the conjunctiva of the upper eye-lid, a pseudo-membrane has formed. It was removed, and the mucous membrane began to bleed. Several other membranes have formed on the mucous membrane near the the larynx (1-2 mm. thick).

April 25.—The condition is about the same. Putrid conjunctivitis and appearance of pseudo-membranes. The membranes around the larynx reappear after removal. A putrid secretion from the right nostril has appeared. The general condition of the fowl is much disturbed, but appetite still exists.

May 2.—Dead.

Post-Mortem: Thin carcass. Mucous membrane in the mouth pale. Between tongue and larynx a cheesy croupous membrane has formed, which is easily removed. On the edge of the larynx, a few thick pseudo-membranes have formed. The laryngeal cavity is almost filled up with pseudo-membraneous, cheese-like masses. The membranes are either located on the mucous membrane, or directly on the cartilage. The mucous membrane is entirely transformed into croupous membranes (see Fig. 10). The upper eye-lid contains a solid cheesy mass, which was attached to the surrounding pyogenous membrane by a few thin fibres. The conjunctiva of the same eye-lid is partly covered with a pseudo-membrane. The mucous membrane of the nostril is soft and covered with a putrid, slimy mass containing much solid cheesy matter. The nasal secretion has an offensive smell.

Fowl No. 47. Was inoculated on the 18th of April in the same manner as fowl No. 46. A putrid conjunctivitis formed. Diphtheritic membranes appeared in the mouth. The general condition was much disturbed at the beginning, but improved after a while. The fowl, however, grew thinner, became badly diseased again, and died the 25th of June. (See Fig. 7.)

Post-Mortem: The eye-lids were swollen, and the whole orbital cavity was filled up with a solid cheesy mass and offensive, stinking matter, the eye having disappeared.

Pigeon No. 29. The 14th of April, two drops of a young bouillon culture of *B. pyocyaneus* were injected under the conjunctiva of the left upper eye-lid. The next day the general condition was very much disturbed, the eye-lid swollen and sticking together. The eye-lids contained much slimy secretion and later two small croupous membranes had formed on the conjunctiva, the conjunctivitis became putrid, and the secretion smelt offensively. Often pseudo-membranes appeared on the conjunctiva. A solid tumor formed in the upper eye-lid. The cornea was turbid. Subsequently, the bird recovered.

Pigeon No. 30. Was inoculated with 1 c.c. culture of *B. pyocyaneus* in the peritoneal cavity. Six hours later, it was dead.

Post-Mortem: The post-mortem examination showed an extended serous putrid peritonitis. The spleen was enlarged and cultures from this organ produced a pure culture of the inoculated bacillus.

From these few experiments with the *B. pyocyaneus*, it is apparent that this bacillus can produce chicken diphtheria in its different localizations in the mouth, eyes, nose, lungs, etc.

The virulence of the *B. pyocyaneus* is very soon lost by growing it in the ordinary culture media, but the virulence can be increased by passing it through several pigeons.

Reviewing the results of our experiments with chickens and pigeons, and comparing them with the symptoms of the natural disease, we conclude that chicken-diphtheria or roup is a complex of putrid processes, especially affecting the mucous membranes and the submucous tissues of the head. We could produce aseptic putrid processes in chickens by inoculation of sterile turpentine oil into the submucous tissue of the eye-lids, and always found the pus in the form of a solid, cheesy mass, which showed no tendency to become soft and liquid. Putrid processes in chickens seem to behave similar to those often found in rabbits, that is, the pus appears as a dry, cheesy, solid mass, not as a fluctuating abscess. (See the submucous and subcutaneous tumors in chicken-diphtheria).

In the cases of a severe putrid catarrh, the pus corpuscles often stick together, with the help of the abnormal secretion, from the mucous membranes or glands (note the jelly-like masses in the eye-lids), and finally become large, cheesy masses, which do not adhere to the diseased mucous membranes. (Cheesy masses in the nose, eye-lids, pleura, and peritoneum). In the mouth cavity, pharynx, larynx, and occasionally on the conjunctiva, the leucocytes, which passed through the mucous membranes, may remain adherent to these. Corresponding to the amount of transuded leucocytes and the degree in which the mucous membranes have been destroyed by them, or by accompanying fibrinous exudations, we observe pseudo-membranes of different severity and size.

The reason the transuded pus corpuscles remain attached to the surface of the mucous membrane in the mouth, and often in the eyes, too, but not in the nose, may be explained as follows: 1. The leucocytes have a natural tendency to stick together. 2. The described processes are very often accompanied by formation of pathological secretions (jelly-masses) in eyes and slimy

saliva, or by fibrinous exudations between the cell elements of the epithelial tissue.

These two circumstances, especially the latter, produce the formation of pseudo-membranes. The third, and perhaps most important, reason is that the mucous membranes in the nostrils possess very many mucous cells, which mix their secretion products with the transudated leucocytes, and remove these from the epithelial surface. The conjunctiva, it is true, is not without mucous cells; but they are relatively scarce. Pharynx and mouth are without mucous cells, and, therefore, pseudo-membranes easily form in these locations.

Besides the two bacilli already described as causing the chicken diphtheria, other causal agents are more or less known; thus, Loeffler described the *Bacillus diphtheriae columbarum*, Loir and Ducloux, the *Bacillus diphtheriae gallinarum* as causing diphtheria in fowls, and proved it by many experiments. Besides, there are protozoa, or corpuscles similar to protozoa claimed by other writers as the cause of fowl diphtheria.

All these results show that diphtheria in fowls cannot be a specific disease like the human one; and, from this point of view, we have to consider the pathological symptoms. For example, the fowl diphtheria in Tunis, as reported by Loir and Ducloux, is differentiated from all other forms by the very acute character of the disease; the presence of the infectious germs in all organs, secretions and excretions; and the easy infection of healthy fowls with any parts of diseased ones.

European and American fowl diphtheria is especially a chronic disease, with very variable virulence, and severity of symptoms, which partly depends on the causal agent.

Roup or fowl diphtheria is generally brought into a healthy flock by a diseased fowl from another place, a fowl having suffered from a chronic attack of the disease (the more common form may be a catarrh of the nostrils or conchae and cella infra-orbitalis) during the summer suddenly becomes very ill again during the late autumn or winter; spreads the germs of the disease and infects the flock.

The third possibility is that in unhygienic locations, fowl diphtheria may appear without any preceding attack or importation of a diseased bird, the causal organism being very ubiquitous, for example, *B. pyocyaneus*. By means of the pathological secretions, the bacilli are spread throughout the whole fowl-yard. These germs may become more virulent by passage through one fowl to another. As the appetite is not generally altered at the commencement of the disease, water and food utensils become infected with the causal organisms, and if these are sufficiently virulent, infection may take place by means of contaminated food or water; but in all cases which have come under our observation, the first symptoms of roup generally appeared after common catarrh of the mucous membranes. The roup germs, therefore, seem to be unable to invade normal mucous membranes, but become a great danger to those affected by common colds. Poultry keepers know very well how exposed chickens and other fowls are to all kinds of colds by sudden changes in

the weather. Young birds and individuals of the finer breeds are especially susceptible.

Fowl Diphtheria usually appears here with the unfavorable weather of the fall and early winter. In one night, a large number of fowls may become affected with catarrh, following which some fowls may become ropy, while others recover in a short time.

The experiments to transfer the disease by means of the pathologic secretions and excretions seems to contradict to a certain extent, our opinion about the way natural infection takes place. To explain this, we must bear in mind the fact that the causal organisms may be present in the pathologic secretions etc., in large numbers, and in a very virulent form, whenever the germs are located on the surface, or in the highest layers of the epithelial membranes (See fowl No. 8, and the result of isolating the bacilli from its pathologic products).

The Roup bacilli are not always localized in one place like the Klebs-Loeffler bacillus, which produces human diphtheria. The organisms causing Roup penetrate into the deeper and submucous tissues, and become the cause of phlegmonous processes and secondary catarrh of the adjacent mucous membranes and glands. In such cases, the roup bacilli are seldom present in the slimy, putrid secretions and pseudo-membranes. Further, the roup bacilli have often entirely disappeared from the pathologic secretions, or are mixed with many other bacterial species. This phenomenon occurs not only in chronic cases of natural infection, but also takes place in fowls which have been inoculated with the roup bacillus.

The pathological products themselves are sufficient, owing to their localization, their consistency, and the histological changes they cause, to produce the continuance of chronic catarrhs. Finally, as already stated the roup bacilli, enclosed in the cheesy masses, lose their vitality.

The fowls experimented with did not suffer from common colds, which must play an important part in the natural disease. As our experiments show, it is easier to infect healthy birds by keeping them a long time with diseased ones (5 out of 10 became diseased) than by direct inoculation of pathologic products on the experimental animals. The explanation of this is that fowls kept with diseased ones for a long time, are exposed to the possibilities of an infection, and at some time during this exposure the natural resistance of the bird is lowered, or some unfavorable condition allows infection to occur.

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